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# HPV Data Set

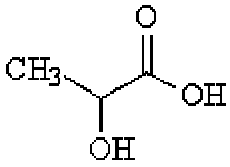
# LACTIC ACID

# CAS # 50-21-5

# Dossier number 50215

# Substance information

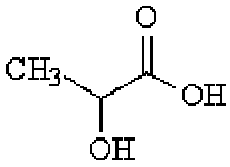
**CAS No.** 50-21-57
**EINECS Name**: lactic acid
**EC No.** 200-018-0
**TSCA Name**: Propanoic acid, 2-hydroxy-, (2S)-
**Molecular Formula** C3H6O3



**IUPAC Name:** LACTIC ACID
**Mol. Weight:** 90
**Physical status:** liquid or white crystals
**Colour:** colourless to slightly yellow
**Odour:** nearly odourless

**Most of the lactic acid nowadays is supplied in the L(+) form, the natural form.**
**PURAC only manufacturers the L(+) form.**
**Substance information for the L(+) lactic acid is as follows:**

**CAS No.** 79-33-4
**EINECS Name**: L(+) lactic acid
**EC No.** 201-196-2
**TSCA Name**: Propanoic acid, 2-hydroxy-, (2S)-
**Molecular Formula** C3H6O3



**IUPAC Name:** L(+) LACTIC ACID
**Mol. Weight:** 90
**Physical status:** liquid or white crystals
**Colour:** colourless to slightly yellow
**Odour:** nearly odourless

**This HPV data file typically contains information about L(+) lactic acid.**

# Chapter 1 Physico-chemical Data

- ***Melting Point***

| | |
|---|---|
| Value: | ≤ 54 degree C |
| Decomposition: | no at < 110 degree C |
| Sublimation: | no |
| Method: | other |
| GLP: | no data |
| Test substance: | 100% crystalline pure product used |

- ***Melting Point of the anhydrous form of the racemic mixture***

Kraft and Dyes found for the racemic lactic acid the melting point 18°C., Borsook, Huffman and Liu reported 16.8°C and more recently Lockwood, Yoder and Zienty reported values of up to 28-33°C. When one of the optically active isomers is present in excess, the melting point is higher; this indicates that racemic lactic acid forms a eutectic mixture.

Ref: ***Lactic Acid: properties and chemistry of lactic acid and derivatives by C.H. Holten, 1971, page 21***

- **Boiling Point / Vapour pressure**

| | |
|---|---|
| Boiling point: | ca. 258 degree C at 1000 hPa |
| Vapour pressure: | ca. .0041 hPa at 20 degree C |
| Decomposition: | yes |
| Method: | other |
| GLP: | no data |
| Remark: | solutions can polymerise on boiling |

It is not possible to determine this value very accurate, because lactic acid tends to polymerise by a polycondensation reaction.
The boiling point of lactic acid at difference vapour pressures according to

- Aspen Database
- PURAC In-house Pure compound database

**Table** Boiling points of lactic acid at different pressures (Aspen Database)

| Pressure (mbar) | Boiling temperature (°C) |
|---|---|
| 1 | 79.6 |
| 5 | 101.9 |

| | |
|---|---|
| 10 | 112.8 |
| 50 | 141.8 |
| 100 | 156.2 |
| 200 | 172.1 |
| 400 | 189.8 |
| 600 | 201.0 |
| 800 | 209.4 |
| 1013 | 216.6 |

The vapour pressures of lactic acid as a function of the boiling temperature according to the Aspen formula (PLXANT).

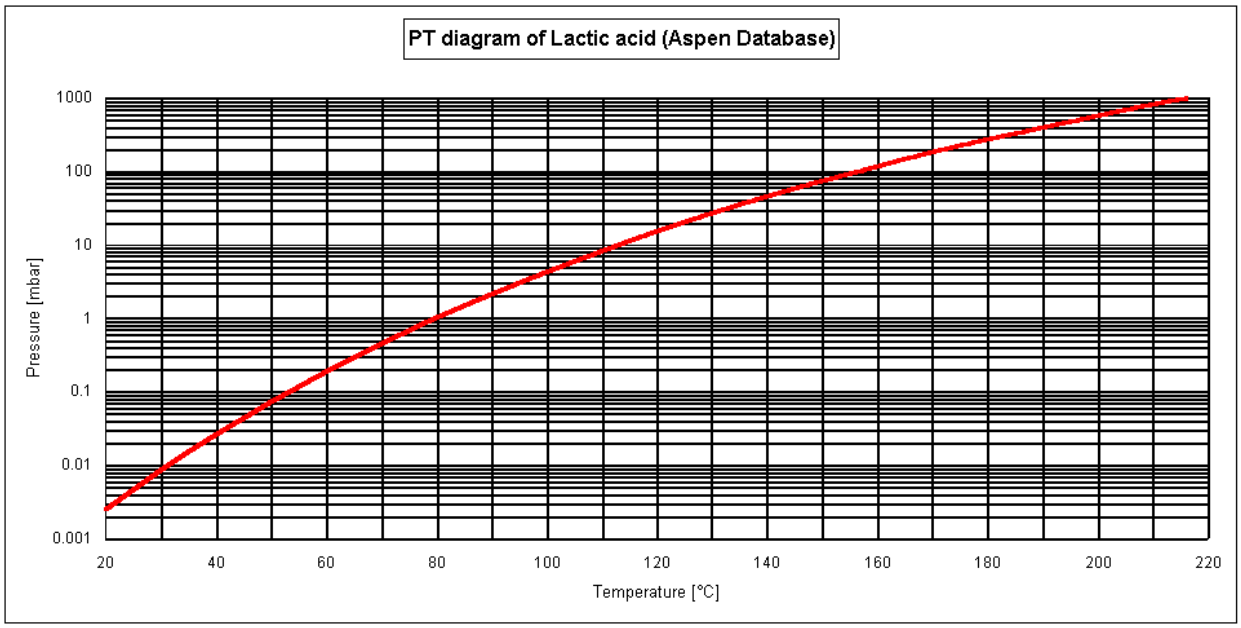
$$PLXANT = e^{C1+\frac{C2}{C3+T}+C4*T+C5*\ln T+C6*T^{C7}}$$

PLXANT : Vapour pressure ($N/m^2$ or Pa)

C1, C2 C3, C4, C5, C6, C7 : Regression coefficients for chemical compound

T : Boiling temperature (K)

**Figure** Vapour Pressure diagram




The regression coefficients are:

C1 = 225.19 C5 = -28.816

C2 = -18757 C6 = 0.000012998

C3 = 0 C7 = 2

C4 = 0

G.P.v.Lieshout measured the vapour pressure of lactic acid between 0 and 180 °C.

**Table** Boiling points of lactic acid at different pressures

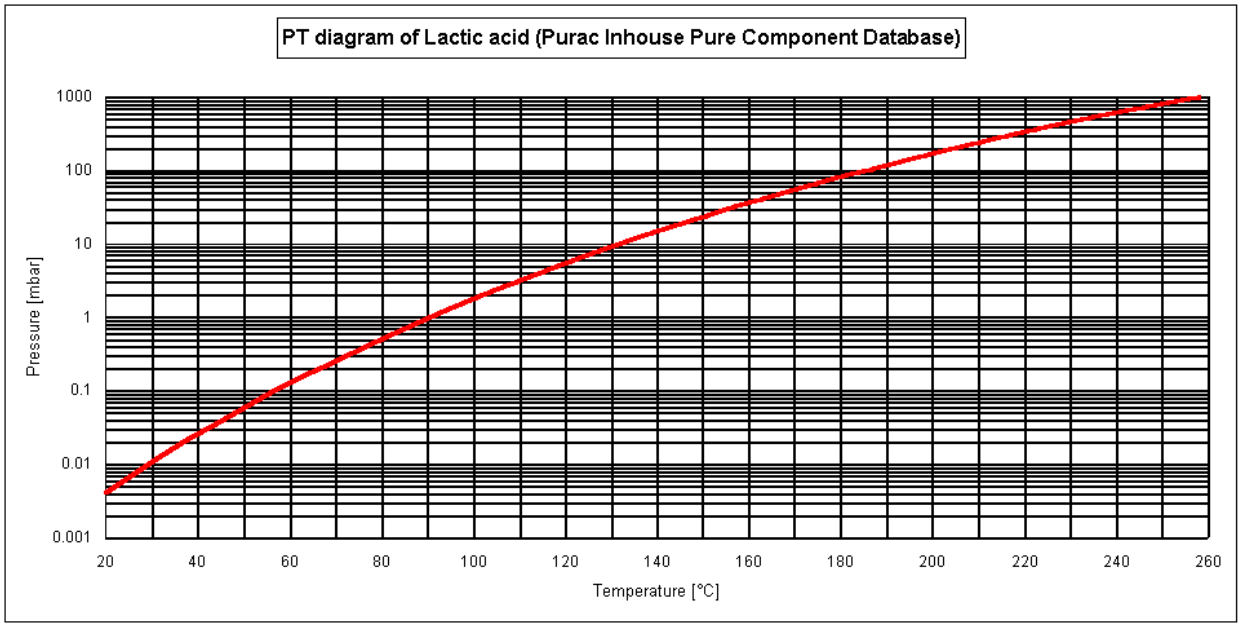
| Temperature (°C) | Vapour Pressure (mbar) |
|---|---|
| 0 | 0.0005 |
| 10 | 0.0015 |
| 20 | 0.0041 |
| 30 | 0.0107 |
| 40 | 0.0258 |
| 50 | 0.0588 |
| 60 | 0.1273 |
| 70 | 0.2629 |
| 80 | 0.5198 |
| 90 | 0.9877 |
| 100 | 1.8093 |
| 110 | 3.2042 |
| 120 | 5.5004 |
| 130 | 9.1736 |
| 140 | 14.8959 |
| 150 | 23.5938 |
| 160 | 36.5163 |
| 170 | 55.3114 |
| 180 | 82.1111 |

The vapour pressures of lactic acid as a function of the boiling temperature were fitted with the Aspen formula (PLXANT). The estimated atmospheric boiling point is about 258 °C.

$$PLXANT = e^{C1+\frac{C2}{C3+T}+C4*T+C5*\ln T+C6*T^{C7}}$$

PLXANT : Vapour pressure ($N/m^2$ or Pa)

C1, C2 C3, C4, C5, C6, C7 : Regression coefficients for chemical compound



: Boiling temperature (K)

Figure: Vapour Pressure diagram
The regression coefficients are:

| | |
|---|---|
| C1 = 32.4649 | C5 = -0.376037 |
| C2 = -8835.5 | C6 = 0 |
| C3 = 0 | C7 = 0 |
| C4 = -0.003654 | |

- **Density**

Type: density
Value: >= 1.2255 g/cm³ at 20 degree C
Method: other
GLP: yes
Test substance: Lactic acid 80%

Holten describes the liquid density of lactic acid solutions in water.
**Table** Liquid Densities of aqueous lactic acid solutions at different temperatures.

| Concentration (wt%) | Temperature (°C) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 10 | 20 | 30 | 40 | 50 | 60 | 70 | 80 | 90 | 100 |
| 0 | 1.0004 | 0.9997 | 0.9979 | 0.9953 | 0.9919 | 0.9878 | 0.9831 | 0.9778 | 0.9720 | 0.9658 | 0.9591 |
| 10 | 1.0269 | 1.0251 | 1.0223 | 1.0188 | 1.0145 | 1.0097 | 1.0042 | 0.9983 | 0.9919 | 0.9851 | 0.9779 |
| 20 | 1.0535 | 1.0505 | 1.0468 | 1.0424 | 1.0373 | 1.0317 | 1.0256 | 1.0190 | 1.0120 | 1.0047 | 0.9970 |
| 30 | 1.0802 | 1.0762 | 1.0715 | 1.0662 | 1.0604 | 1.0540 | 1.0472 | 1.0400 | 1.0325 | 1.0246 | 1.0164 |
| 40 | 1.1069 | 1.1019 | 1.0964 | 1.0902 | 1.0836 | 1.0766 | 1.0692 | 1.0614 | 1.0533 | 1.0449 | 1.0363 |
| 50 | 1.1333 | 1.1275 | 1.1211 | 1.1142 | 1.1069 | 1.0992 | 1.0912 | 1.0829 | 1.0743 | 1.0655 | 1.0564 |
| 60 | 1.1591 | 1.1524 | 1.1453 | 1.1377 | 1.1299 | 1.1216 | 1.1131 | 1.1043 | 1.0953 | 1.0861 | 1.0766 |
| 70 | 1.1835 | 1.1762 | 1.1685 | 1.1604 | 1.1521 | 1.1434 | 1.1345 | 1.1254 | 1.1160 | 1.1065 | 1.0967 |
| 80 | 1.2061 | 1.1983 | 1.1902 | 1.1818 | 1.1731 | 1.1642 | 1.1550 | 1.1456 | 1.1360 | 1.1263 | 1.1163 |
| 90 | 1.2263 | 1.2183 | 1.2100 | 1.2014 | 1.1925 | 1.1834 | 1.1741 | 1.1646 | 1.1550 | 1.1451 | 1.1351 |
| 95 | 1.2354 | 1.2274 | 1.2190 | 1.2104 | 1.2015 | 1.1924 | 1.1831 | 1.1736 | 1.1639 | 1.1541 | 1.1441 |
| 100 | 1.2438 | 1.2357 | 1.2274 | 1.2188 | 1.2099 | 1.2008 | 1.1916 | 1.1821 | 1.1725 | 1.1627 | 1.1527 |

**Liquid Density of <u>pure lactic acid</u>**

The liquid density of pure lactic acid at difference temperatures according to:

- Aspen Database
- PURAC In-house Pure Component Database

**Table** Densities of lactic acid at different temperatures (ASPEN Database)

| Temperature (°C) | Density (g/ml) |
|---|---|
| 20 | 1.2255 |
| 30 | 1.2163 |
| 40 | 1.2069 |
| 50 | 1.1974 |
| 60 | 1.1878 |
| 70 | 1.1780 |
| 80 | 1.1681 |
| 90 | 1.1580 |
| 100 | 1.1478 |
| 110 | 1.1374 |
| 120 | 1.1268 |
| 130 | 1.1160 |
| 140 | 1.1051 |
| 150 | 1.0939 |
| 160 | 1.0825 |

The liquid densities of lactic acid as a function of the temperature according to the Aspen equation for liquid density (DNLDIP):

$$DNLDIP = \frac{C1}{C2^{1+(1-\frac{T}{C3})^{C4}}}$$

DNLDIP : Liquid density (kmole/$m^3$)
C1, C2 C4 : Regression coefficients for chemical compound
C3 : Critical temperature (K)
T : Temperature (K)
The regression coefficients are:
C1 = 0.9798 C3 = 675
C2 = 0.24593 C4 = 0.2333

**Liquid density diagram**

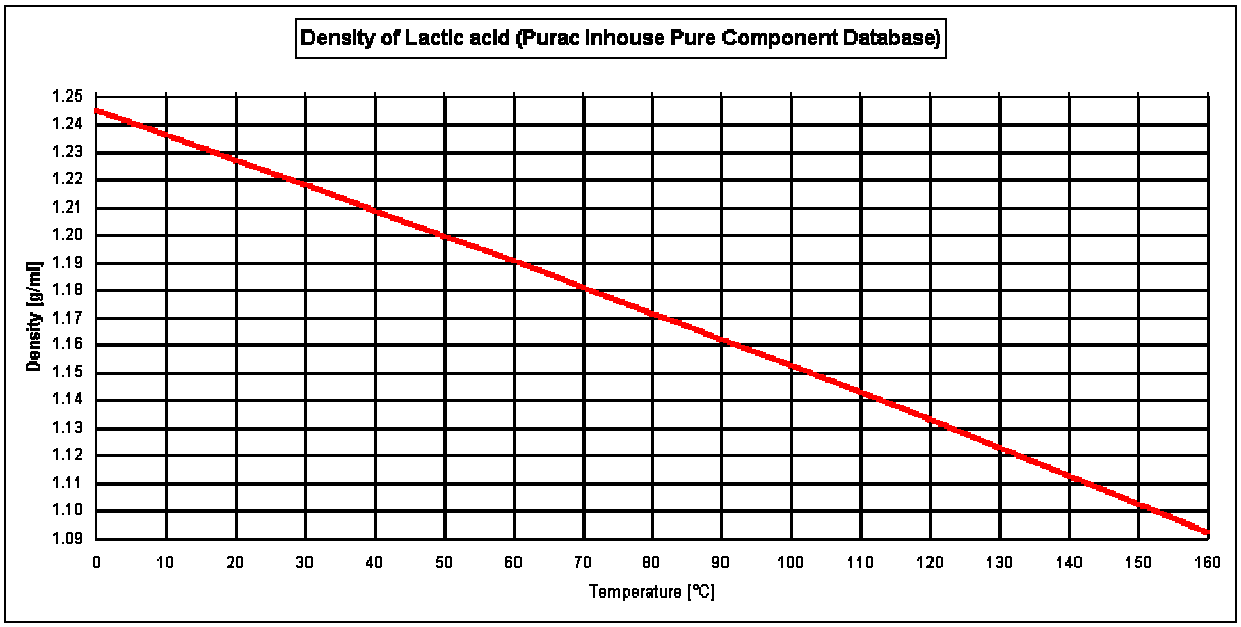



**Table** Densities of lactic acid at different temperatures (Purac In-house Pure Component Database)

| Temperature (°C) | Density (g/ml) |
|---|---|
| 0 | 1.2453 |
| 10 | 1.2364 |
| 20 | 1.2274 |
| 30 | 1.2183 |
| 40 | 1.2092 |
| 50 | 1.2000 |
| 60 | 1.1907 |
| 70 | 1.1813 |
| 80 | 1.1719 |
| 90 | 1.1623 |
| 100 | 1.1527 |
| 110 | 1.1429 |
| 120 | 1.1330 |
| 130 | 1.1231 |
| 140 | 1.1130 |
| 150 | 1.1028 |
| 160 | 1.0924 |

The liquid densities of lactic acid as a function of the temperature according to the Aspen equation for liquid density (DNLDIP):

$$DNLDIP = \frac{C1}{C2^{1+(1-\frac{T}{C3})^{C4}}}$$

DNLDIP : Liquid density (kmole/$m^3$)

C1, C2 C4 : Regression coefficients for chemical compound
C3 : Critical temperature (K)
T : Temperature (K)
The regression coefficients are:
C1 = 1.61248 C3 = 729.9
C2 = 0.314479 C4 = 0.328317

- ***Partition Coefficient***

Partition Coeff.: octanol-water
log Pow: ca. -.62 at 20 degree C
Method: OECD Guide-line 117 "Partition Coefficient (n-octanol/water), HPLC Method"
Year: 1987
GLP: yes

- ***Solubility in water***

Value: ca. 100 vol% at 25 degree C
pH value: ca. 1.2
pKa: 3.68 at 25 degree C
Descr.: of very high solubility
Method: other
GLP: no data
Deg. product: not measured
Stable: yes
Remark: completely soluble at 25 degrees C

J.v.Krieken determined the phase diagram of lactic acid/ water. Due to practical problems the diagram wasn't completed, but the missing part was extrapolated.

**Table** Solubility of monomeric lactic acid in water

| Temperature (°C) | Lactic acid (wt%) |
|---|---|
| -20 | 65.9 |
| -15 | 68.8 |
| -10 | 71.5 |
| -5 | 74.2 |
| 0 | 76.7 |
| 5 | 78.6 |
| 10 | 81.3 |
| 15 | 83.5 |
| 20 | 86.1 |
| 25 | 87.6 |
| 35 | 92.7 |
| 40 | 95.1 |

**Table** Freezing point data of monomeric lactic acid in water

| Temperature (°C) | Lactic acid (wt%) |
|---|---|

| | |
|---|---|
| 0 | 0 |
| -5 | 18.6 |
| -10 | 32.5 |
| -15 | 41.9 |
| -21 | 51.7 |

The eutectic point of lactic acid/water was calculated by extrapolation and is ca. 61.7 wt% lactic acid and -27.1 °C.

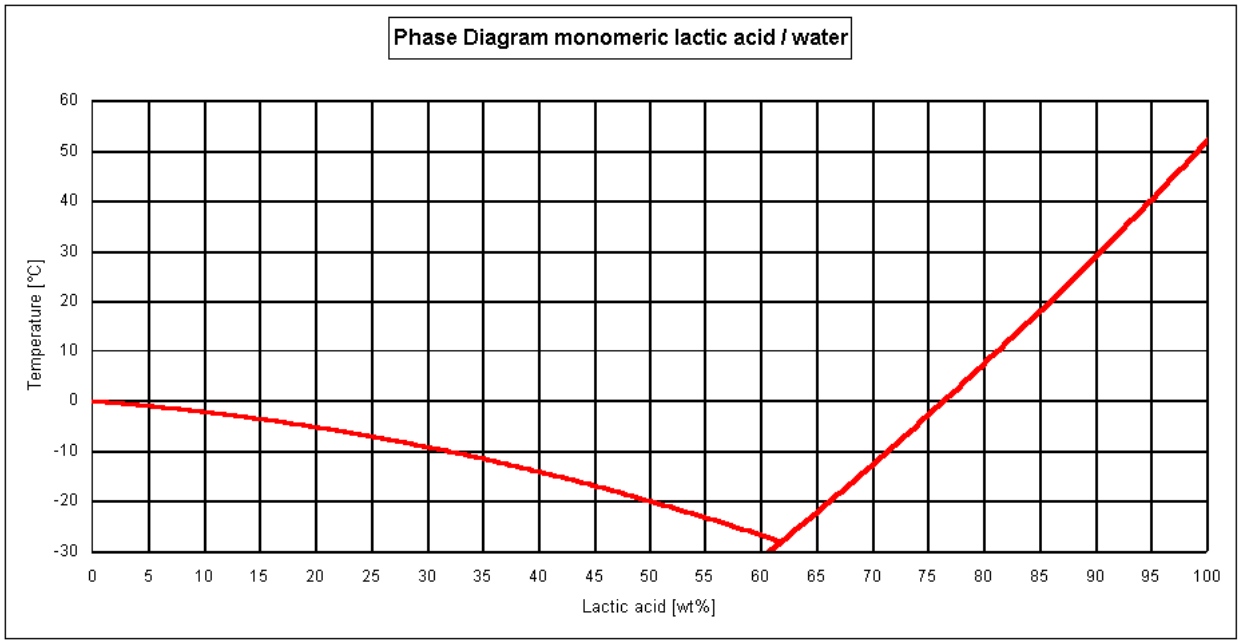



**Figure** Phase Diagram

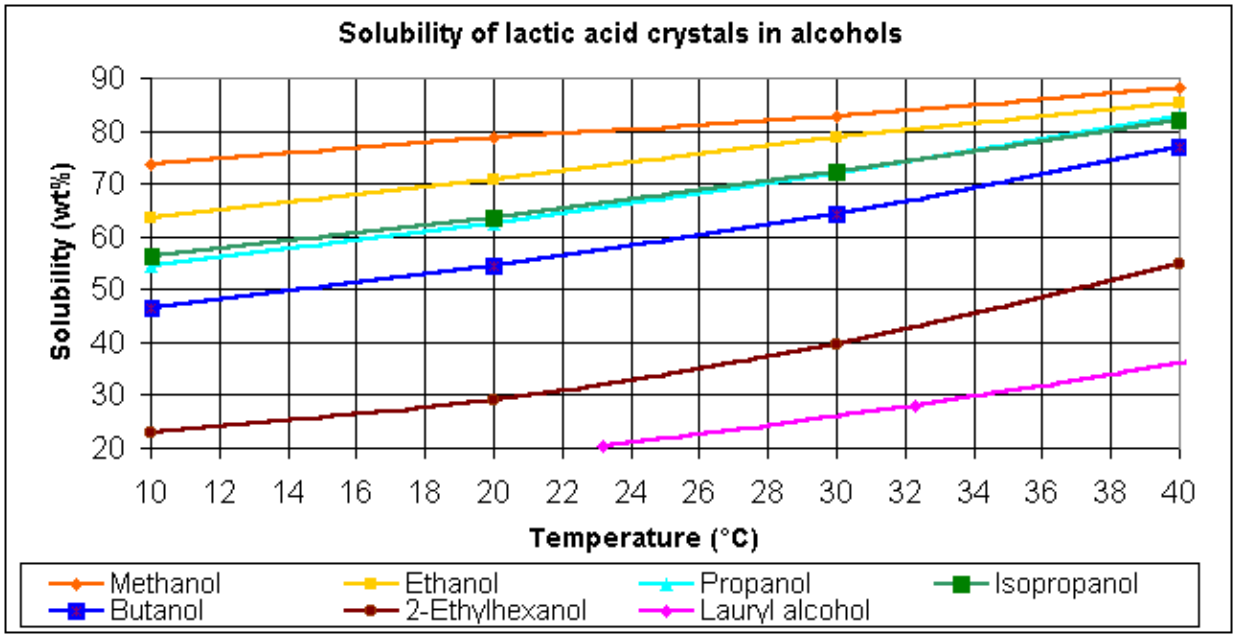
### Solubility of lactic acid in other solvents

J.v. Krieken investigated the solubility of pure lactic acid in a range of solvents by stirring the specific solvent with an excess of (S)-lactic acid crystals at a specific temperature. After separation of the solids from the liquid, the clear liquid was analysed on free acidity.

**Table** solubility of lactic acid in a range of solvents

| Solvent | Solubility (wt%) of solution | | | |
|---|---|---|---|---|
| | 10°C | 20°C | 30°C | 40°C |
| Methanol | 73.8 | 78.6 | 82.8 | 88.1 |
| Ethanol | 63.6 | 70.9 | 78.7 | 85.2 |
| 1-Propanol | 54.5 | 62.4 | 71.7 | 82.7 |
| 2-Propanol | 56.1 | 63.4 | 72.2 | 82.2 |
| 1-Butanol | 46.3 | 54.5 | 64.3 | 77.1 |
| 2-Ethyl-1-hexanol | 22.9 | 29.0 | 39.6 | 54.9 |
| Cyclohexane | | | | 0.04 |
| Hexane | < 0.01 | < 0.01 | < 0.01 | 0.02 |

| | | | | |
|---|---|---|---|---|
| Toluene | 0.06 | 0.11 | 0.24 | 0.50 |
| Ethyl lactate | 37.0 | 45.9 | 57.2 | 72.8 |
| Butyl lactate | 27.7 | 35.7 | 47.3 | 64.8 |
| 2-Ethylhexyl lactate | 15.5 | 20.9 | 30.9 | 46.7 |
| Ethyl acetate | 27.1 | 39.9 | 56.2 | 72.9 |
| Diethyl ether | 23.8 | 38.7 | 59.8 | |
| Diisopropyl ether | 6.4 | 9.2 | 15.9 | 44.4 |
| Tetrahydrofuran | 58.4 | 65.1 | 72.4 | 82.9 |
| Dichloromethane | 0.59 | 1.02 | 2.7 | |
| Chloroform | 0.31 | 0.67 | 1.67 | 59.7 |
| 2-Butanone | 40.7 | 52.9 | 67.3 | 81.6 |
| Acetone | 53.4 | 61.4 | 71.5 | 82.9 |




- ***Viscosity***

Test type: other: ASPEN database ; PURAC internal
Value: ca. 53.52 mPa s (dynamic) at 20 degree C

The liquid viscosity of lactic acid at difference temperatures according to

- Aspen Database
- PURAC In-house Pure Component Database

The liquid viscosity of aqueous lactic acid solutions in water

- 0 - 88 wt% of Lactic Acid
- 90 - 110 wt% Lactic Acid

**Table** Viscosity of lactic acid at different temperatures (Aspen Database)

| Temperature (°C) | Viscosity (cP) |
|---|---|
| 20 | 53.52 |
| 30 | 33.26 |
| 40 | 21.29 |
| 50 | 14.01 |
| 60 | 9.44 |
| 70 | 6.51 |
| 80 | 4.59 |
| 90 | 3.29 |
| 100 | 2.40 |
| 110 | 1.78 |
| 120 | 1.34 |
| 130 | 1.03 |
| 140 | 0.79 |
| 150 | 0.62 |
| 160 | 0.49 |

The liquid viscosity of lactic acid as a function of the temperature according to the Aspen equation for liquid viscosity (MULDIP):

$$MULDIP = e^{C1+\frac{C2}{T}+C3*\ln T+C4*T^{C5}}$$

MULDIP : Liquid viscosity ($N*s/m^2$ or Pa.s)
C1, C2 C3, C4, C5 : Regression coefficients for chemical compound
T : Temperature (K)
The regression coefficients are:
C1 = -14.403
C2 = 4097.9
C3 = -0.4407
C4 = 0
C5 = 0

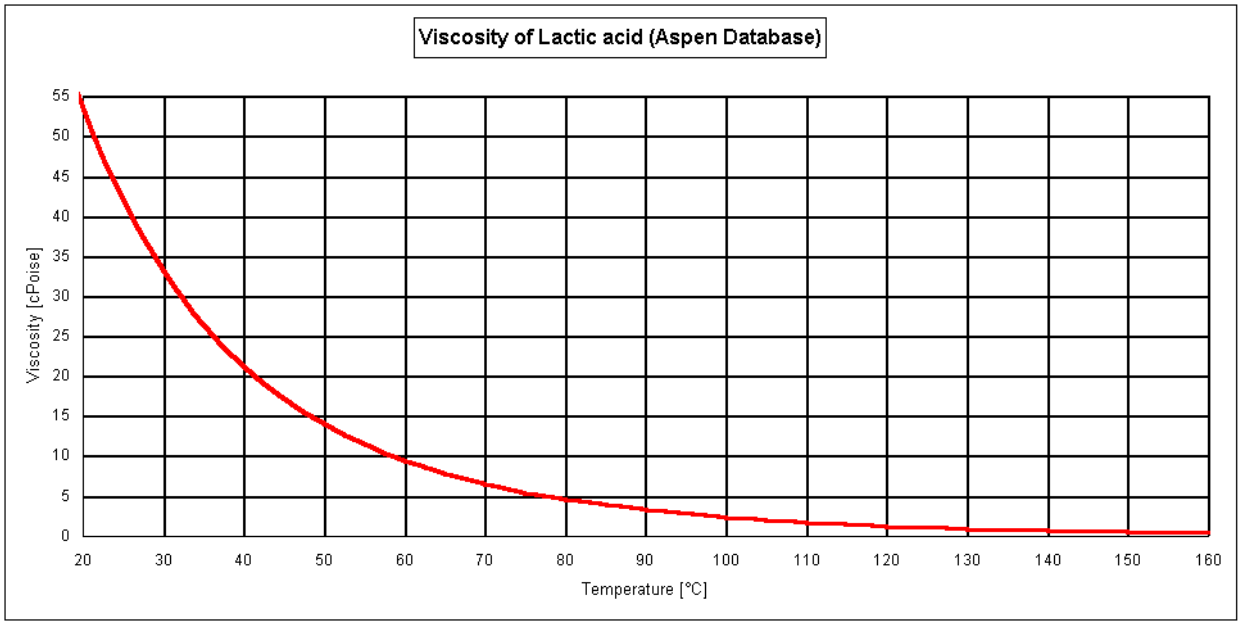


**Figure**: Liquid Viscosity diagram

Table 12 Viscosity of lactic acid at different temperatures (Purac In-house Pure Component Database) [2]

| Temperature (°C) | Viscosity (cP) |
|---|---|
| 20 | 1342.33 |
| 30 | 579.14 |
| 40 | 280.59 |
| 50 | 150.73 |
| 60 | 88.80 |
| 70 | 56.81 |
| 80 | 39.14 |
| 90 | 28.83 |
| 100 | 22.54 |

The liquid viscosity of lactic acid as a function of the temperature according to the Aspen equation for liquid viscosity (MULDIP):

MULDIP: Liquid viscosity ($N*s/m^2$ or Pa.s)
C1, C2, C3, C4, C5: Regression coefficients for chemical compound
T: Temperature (K)

The regression coefficients are:

C1: 421.094
C2: 25091.4
C3: 59.1119
C4: 0
C5: 0

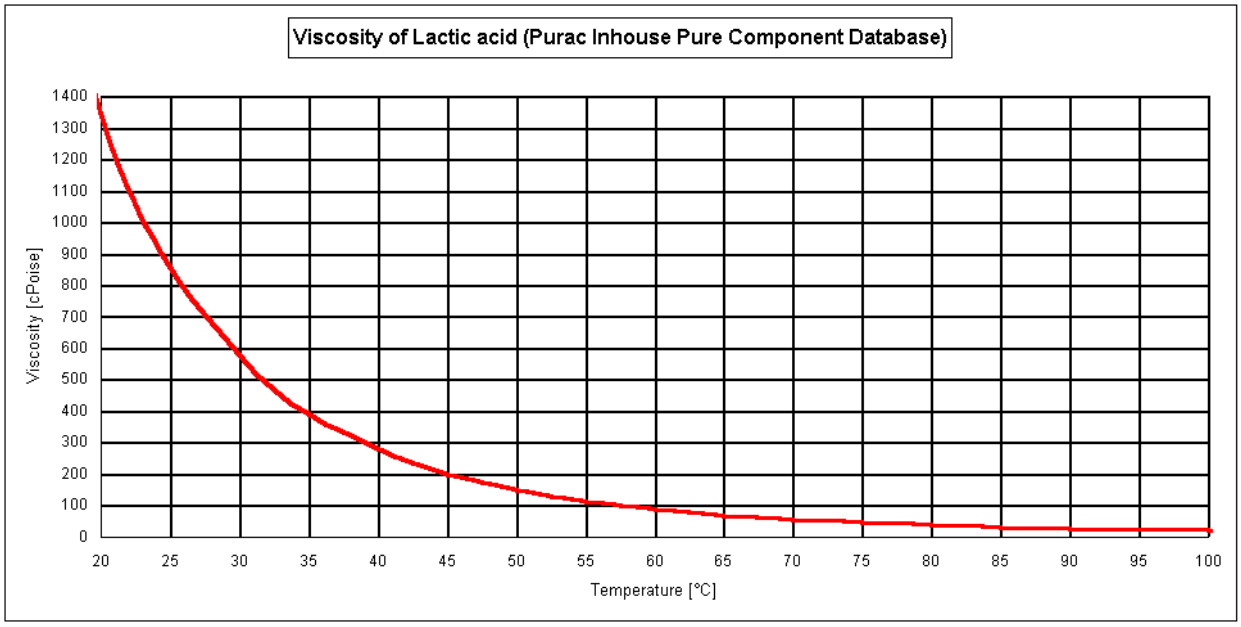



**Figure**: Liquid Viscosity diagram

## *References Chapter 1: Physico chemical data*


- National Chemical Inventories
  Coden NCINF5
  ISSN: 1089-6279

- Handbook of Chemistry and Physics
  WEAST
  66th Edition

- ASPEN database

- PURAC internal databank

- ***Photodegradation***

The photochemical oxidisation of lactic acid is discussed in "Lactic acid properties and chemistry of lactic acid and derivatives by C.H. Holten (1971)". The first observation that lactic acid is photosensitive was made in 1910 by Berthelot and Gaudechon, who irradiated calcium lactate and ethyl lactate with ultraviolet rays. They observed decomposition with the formation of gas containing carbon monoxide, carbon dioxide, hydrogen and methane.

Recognized method, i.e. OECD: Modelling conducted, no guideline studies used.

Method: Estimated Programs Interface (EPIWIN V3.05, Atmospheric Oxidation Program v 1.90). Model executed in October 2002.

Results / observations: The AOP component of EPIWIN was used to calculate the rate of photodegradation for L(+) lactic acid.

SMILES : O=C(O)C(O)C
CHEM : Propanoic acid, 2-hydroxy-, (S)-
MOL FOR: C3 H6 O3
MOL WT : 90.08

---------- SUMMARY (AOP v1.90): HYDROXYL RADICALS ----------

| | | |
|---|---|---|
| Hydrogen Abstraction | = | 5.2598 E-12 cm3/molecule-sec |
| Reaction with N, S and –OH | = | 0.6600 E-12 cm3/molecule-sec |
| Addition to Triple Bonds | = | 0.0000 E-12 cm3/molecule-sec |
| Addition to Olefinic Bonds | = | 0.0000 E-12 cm3/molecule-sec |
| Addition to Aromatic Rings | = | 0.0000 E-12 cm3/molecule-sec |
| Addition to Fused Rings | = | 0.0000 E-12 cm3/molecule-sec |
| OVERALL OH Rate Constant | = | 5.9198 E-12 cm3/molecule-sec |
| HALF-LIFE | = | 1.807 Days (12-hr day; 1.5E6 OH/cm3) |
| HALF-LIFE | = | 21.682 Hrs |

---------- SUMMARY (AOP v1.90): OZONE REACTION ----------

****** NO OZONE REACTION ESTIMATION ******
(ONLY Olefins and Acetylenes are Estimated)

Experimental Database: NO Structure Matches

- ***Stability in Water***

Lactic Acid (88% and 60% aqueous solutions) were investigated.
"The kinetics of degradation of Lactic Acid was done at elevated temperature, since the decomposition rates of lactic acid, (..) were too slow to obtain kinetic data within

reasonable time. At the condition studied (25, 40, 80 and 120 degrees centigrade) the decompositions of these compounds followed apparent first order kinetics because the mean correlation coefficient was above 0.980.
Lactic acid was very stable in aqueous solutions at 80 degrees centigrade (less than 30% decomposition after 175 at 80 degrees) and degradation was not different when combined with the various excipients tested. The shelf lives determined for lactic acid ranged from 79 years when combined with isopropyl palmitate to 98 years when combined with sorbic acid.

- ***Transport between Environmental Compartments***

Type: other: see free texts RM.
Remark: Lactic acid is not volatile and it has a high biodegradation rate. Therefor transport between compartments is no issue for this compound.

No experimental data are available on fugacity. However, calculation using the sotware program EPI Suite version 3.1 gave the following information which supports the statement given above:

| Level III Fugacity Model: | | | |
|---|---|---|---|
| | Mass Amount (%) | Half-Life (hr) | Emissions (kg/hr) |
| | | | |
| Air | 3.16 | 43.4 | 1000 |
| Water | 46.3 | 208 | 1000 |
| Soil | 50.5 | 208 | 1000 |
| Sediment | 0.0691 | 832 | 0 |
| | | | |
| Persistence Time: 222 hr | | | |

- **Biodegradation**

Value 50% degradation after 5 days and 67% after 20 days.
Breakdown Product: It is to be expected that L(+) lactic acid will be taken up into the metabolism of the bacteria in the activated sludge. Degradation products will therefor be $CO_2$ and $H_2O$.
Method: BOD (Biochemical Oxygen Demand) and COD (Chemical Oxygen Demand) determinations were carried out for L(+) lactic acid usig the method described in teh Dutch guidenlines "water determination of biochemical oxgen demand after n days (BODn)" (NEN 6634) and "Water determination of chemical oxygen demand (COD" (NEN 6633) respectively, these methods are similar to those referred in the EC Test Guidelines C.8 and C.9 Two concentrations (2 mg/L and 4 mg/L) were tested.
An oculum was prepared from activated sludge. Its microbial activity appeared to be sufficient although the control substance glucose and glutamic acid had a BOD5 of slightly less than the required value of 4.00 ± 0.75 mg $O_2$/L

## *References Chapter 2: Environmental fate and pathways*


- Lactic Acid: properties and chemistry of lactic acid and derivatives by C.H. Holten, 1971, page 38

- TNO report R 92/018: BOD and COd of L(+) lactic acid according to EC test guidelines C.8 and C.9

- The ecotoxicity and the biodegradability of lactic acid, alkyl esters and lactate salts
  C.T. Bowmer et.al.
  Chemosphere, volume 37, No 7, pp 1317 - 1333, 1998

- Stability of lactic acid and glycolic acid in aqueous systems subjected to acid hydrolysis and thermal decomposition.
  M.M. de Villiers et.al.
  Journal of the society of cosmetic chemists, 48, 165-174 (August 1998)

- Handbook of Chemistry and Physics
  WEAST
  66th Edition

# Chapter 3 Ecotoxicity

- ***Acute/Prolonged Toxicity to Fish***

| | | |
|---|---|---|
| - | Species: | Brachydanio rerio (2.1 ±0.17 cm and 0.07 ±0.018 g) |
| | Report number: | TNO R 91/295 |
| | Type: | Semistatic |
| | Exposure period: | 48 hour(s) |
| | Test substance: | 80% L(+) Lactic Acid |
| | Nominal concentrations: | 0, 100, 180, 320, 560 and 1000 mg/l |
| | Analytical monitoring: | yes |
| | 48h LC50: | = 320 - calculated |
| | Limit Test: | no |
| | Method: | OECD Guideline 203 "Fish, Acute Toxicity Test" |
| | Water temperature: | Between 24.0 and 24.7 °C |
| | Water hardness: | About 220 mg/l expressed as $CaCO_3$. |
| | Alkalinity: | Unknown |
| | Total Organic content: | 2.0 mg/l |
| | Dissolved oxygen: | > 7.3 mg/l |
| | pH levels: | During test between 3.2 and 8.2 |
| | Year: | 1992 |
| | GLP: | yes |
| | Remark: | test solutions are not neutralised. It is more than likely that the low pH value affected the survival of the fishes. |

- Species: Brachydanio rerio (2.1 ±0.17 cm and 0.07 ±0.018 g)
  Report number: TNO R 91/295
  Type: Semistatic
  Exposure period: 96 hour(s)
  Test substance: 80% L(+) Lactic Acid
  Nominal concentrations: 0, 100, 180, 320, 560 and 1000 mg/l
  Analytical monitoring: yes
  96h NOEC: = 320 mg/l - measured/nominal
  96h LC50: = 320 mg/l - calculated
  96h LC100: = 560 mg/l - calculated
  Method: OECD Guideline 203 "Fish, Acute Toxicity Test"
  Water temperature: Between 24.0 and 24.7 °C
  Water hardness: About 220 mg/l expressed as $CaCO_3$.
  Alkalinity: Unknown
  Total Organic content: 2.0 mg/l
  Dissolved oxygen: > 7.3 mg/l
  pH levels: During test between 3.2 and 8.1
  Year: 1992
  GLP: yes
  Remark: test solutions are not neutralised. It is more than likely that the low pH value affected the survival of the fishes.

- Species: Lepomis macrochirus (24± 2.3 mm and 0.37 ± 0.15 g)
  Report number: Analytical Bio Chemistry lab # 32146
  Type: Static
  Exposure period: 96 hour(s)
  Test substance: 80% L(+) Lactic Acid
  Nominal concentrations: 0, 56, 100, 180, 320 and 560 mg/l
  Analytical monitoring: yes
  NOEC: = 56 mg/l - measured/nominal
  24 h LC50: = 140 mg/l - measured/nominal
  48 h LC50: = 130 mg/l - measured/nominal
  96h LC50: = 130 mg/l - measured/nominal
  Limit Test: yes
  Method: :
  Ten fish were exposed to each test concentration and control.
  The procedures for static bioassay described in (1) and (2) below were used in this experiment.
  (1) Committee on methods for Toxicity Tests with Aquatic Organisms (C.E. Stephan chairman). 1975. Methods for acute toxicity tests with fish, macro invertebrates and amphibians.
  Environmental Protection Agency, Ecological Research Series EPA 660/3-75-009, April 1975; 61p
  Water temperature: 22 °C ( ±1,0 )
  Water hardness: 40 - 45 mg/l as $CaCO_3$.
  Alkalinity: 30 - 35 mg/l as $CaCO_3$.
  Dissolved oxygen: 9.0 mg/l (at time = zero)
  pH levels: During test between 7.2 – 7.6

  (2) American Public Health Association. 1980. Standard methods for the examination of water and wastewater. 15th ed. Washington DC 1134p.
  Year: 1984
  GLP: yes

| | |
|---|---|
| Species: | Salmo gairdneri (42 ± 3.4 mm and 1.09 ± 0.28 g) |
| Report number: | Analytical Bio Chemistry lab # 32147 |
| Type: | Static |
| Exposure period: | 96 hour(s) |
| Test substance: | 80% L(+) Lactic Acid |
| Nominal concentratins: | 0, 32, 56, 100, 180 and 320 mg/l |
| Analytical monitoring: | yes |
| NOEC: | = 56 mg/l - measured/nominal |
| 24h LC50: | = 150 mg/l - measured/nominal |
| 48 h LC50: | = 130 mg/l - measured/nominal |
| 96h LC50: | = 130 mg/l - measured/nominal |

Method:

The static fish bioassay was conducted in five gallon glass vessels containing 15 litres reconstituted water. The study was conducted at the nominal concentrations of 32, 56, 100,180 and 320 mg/l. Ten fish were exposed to each test concentration and control.

| | |
|---|---|
| Water temperature: | 12 °C ( ±1.0 ) |
| Water hardness: | 40 - 45 mg/l as $CaCO_3$. |
| Alkalinity: | 30 - 35 mg/l as $CaCO_3$. |
| Dissolved oxygen: | During test between 6.1 – 9.2 mg/l |
| pH levels: | During test between 7.2 – 7.6 |

- ***Acute Toxicity to Aquatic Invertebrates***

| | |
|---|---|
| - Species: | Daphnia magna (less than 24 hours old) |
| Report number: | TNO report R 91/294 |
| Type: | Static |
| Exposure period: | 48 hour(s) |
| Test substance: | 80% L(+) Lactic Acid |
| Nominal concentrations: | 0, 32, 56, 100, 180, 320 and 560 mg/l |
| Analytical monitoring: | yes |
| 48h NOEC: | = 180 mg/l - calculated |
| 48h EC50: | = 240 mg/l - calculated |
| 48h EC100: | = 320 mg/l - calculated |
| 24h EC 50: | = 240mg /l - calculated |
| Limit Test: | no |
| Method**:** | OECD Guide-line 202 |
| Water temperature: | 19.9 °C ( ±1 ) |
| Water hardness: | 220 mg/l as $CaCO_3$. |
| Alkalinity: | Unknown |
| Total organic carbon: | 2.0 mg/l |
| Dissolved oxygen: | > 7.9 mg/l |
| pH levels: | During test between 3.6 – 8.2 |
| Year: | 1992 |
| GLP: | yes |

Remark:
test solutions are not neutralised. It is more than likely that the low pH values affected the mobility of the daphnia's.

- Species: Daphnia magna (less than 24 hours old)
  Report number: Analytical Bio Chemistry lab # 32148
  Type: Static
  Exposure period: 48 hour(s)
  Test substance: 80% L(+) Lactic Acid
  Nominal concentations: 0, 100, 180, 320, 560 and 1000 mg/l
  Analytical monitoring: yes
  NOEC: = 320 mg/l - measured/nominal
  LC50 48h : = 750 mg/l - measured/nominal
  Limit Test: yes

  Method:
  Five concentrations in duplicate of the test compound with ten Daphnia per 250 ml glass beaker were used. The concentrations were a logarithmic series ranging from 100 to 1000 mg/l and included a control. The procedure for static bioassay as described in (1) and (2) below were used.

  (1) Methods of acute toxicity with fish, Macro invertebrates and Amphibians. Stephan, CE, chairman. 1975.
  Committee on Methods for toxicity tests with aquatic organisms. US EPA Ecol. Res. Ser. 660/3-75009.
  (2) American Public Health Association. 1980. Standard methods for the examination of Water and wastewater. 15th ed. Washington DC. 1134p.
  Water temperature: 20 °C ( ±2 )
  Water hardness: 225 - 275 mg/l as $CaCO_3$.
  Alkalinity: 325 - 375 mg/l as $CaCO_3$.
  Dissolved oxygen: 7.1 – 8.5 mg/l (77 – 92 percent saturation at 20°C)
  pH levels: During test between 4.3 – 8.5
  Year 1984
  GLP: yes

- ***Toxicity to Aquatic Plants e.g. Algae***

- Report number: TNO 92/009
  Species: Selenastrum capricornutum (Algae)
  Endpoint: growth rate
  Exposure period: 70 hour(s)
  Test substance**:** 80% L(+) neutralised L(+) lactic acid
  Analytical monitoring: yes
  NOEC: = 1.9 mg/l - calculated
  EC10: = 2.3 mg/l - calculated
  EC50: = 3.5 mg/l - calculated
  EC90 : = 5.4 mg/l - calculated
  Method: OECD Guideline 201 "Algae, Growth Inhibition Test"
  Water temperature: 23 °C ( ±1 )
  Lighting conditions: 120 ± 20% µmol.$S^{-1}$.$m^{-2}$.
  Composition of Growth medium:

| | |
|---|---|
| $NH_4Cl$ | 15 mg/l |
| $MgCL_2.6H_2O$ | 12 mg/l |
| $CaCl_2.2H_2O$ | 18 mg/l |
| $MgSO_4.7H_2O$ | 15 mg/l |
| $KH_2PO_4$ | 1.6 mg/l |
| $FeCl_3.6H_2O$ | 80 µg/l |
| $Na_2EDTA.2H_2O$ | 100 µg/l |
| $H_3BO_3$ | 185 µg/l |
| $MnCl_2.4H_2O$ | 415 µg/l |
| $ZnCl_3$ | 3 µg/l |
| $CoCl_2.6H_2O$ | 1.5 µg/l |
| $CuCl_2.2H_2O$ | 0.01 µg/l |
| $Na_2MoO_4.2H_2O$ | 7 µg/l |
| $NaHCO_3$ | 150 µg/l |

  The pH of this medium after equilibration with air is approximately 8.

  Water hardness: Not applicable
  Dissolved oxygen: flasks were shaken (100 rpm); no data on dissolved oxygen.
  pH levels: During test between 3.6 – 8.2
  Year: 1992
  GLP: yes

- ***Toxicity to Micro-organisms e.g. Bacteria***

- Type: other: laboratory incubations
  Species: Escherichia coli (Bacteria)
  Exposure period: 20 minute(s)
  EC100 : = 15 - measured/nominal
  Test substance`: combinations of 1.0-1.5% lactic acid with 0.1% sodium benzoate, or 0.1% hydrogen peroxide, or 0.005% glycerol monolaurate.
  Result:
  At 22C complete inactivation of E. coli O157:H7 was observed after 20 min. of exposure to 1.5% lactic acid plus 0.1% hydrogen peroxide.
  Conclusion:
  The mentioned treatment could potentially be used to inactivate or reduce E. coli O157:H7 populations on raw products

- Type: other: laboratory incubations on lean beef muscle discs
  Species: other bacteria: Listeria monoccytogenes, Yersinia enterocolitica, Salmonella typhimurium, E.coli, Campylobacter jejuni, Staphylococcus aureus, Pseudomonas fragi, Brochotrix thermosphacta.
  Exposure period: 0 minute(s)
  Remark: Acid temperature (20 & 50 C) and concentration (1%, 3%) and initial numbers of contaminating bacteria (log CFU/cm2 of 3-6) were the variables studied.
  Result: The bactericidal efficacy of lactic acid was often distinct for each organism. Bacterial numbers were maximally reduced with 3% acid at 55C. S.aureus: 1.4 log cycle; P. fragi: 2.3 log cycle; B. thermosphacta: 2.8 log cycle reduction.

# *References Chapter 3: Ecotoxicity*

- Acute / Prolonged toxicity to fish


- TNO report R 91/295; The acute toxicity of L(+)-lactic acid to Brachydanio Rerio (OECD 203).

- Acute toxicity of L(+) lactic acid to Rainbow Trout (Salmo Gairdneri)
  Analytical Biochemistry Laboratories Inc.
  Columbia, MO
  1984

- Acute toxicity of L(+) lactic acid to Bluegill Sunfish (Lepomis macrochirus)
  Analytical Biochemistry Laboratories Inc.
  Columbia, MO
  1984


- Acute toxicity to aquatic invertebrates


- TNO-report R 91/ 294; The acute toxicity of L(+)-lactic acid to Daphnia Magna (OECD 202, 48h).

- Acute toxicity of L(+) lactic acid to Daphnia Magna
  Analytical Biochemistry Laboratories Inc.
  Columbia, MO
  1984


- Toxicity to aquatic plants e.g. algae


- TNO report R 92/009 ; Effect of L(+)-lactic acid on the growth of the alga Selenastrum Capricornutum (OECD 201).


- Toxicity to micro-organisms e.g. bacteria


- Food Microbiology 16: 75-82 (1999), Venkitanarayanan K.S.,
  Zhao T., Doyle M.P., "Inactivation of E.coli 0157:H7 by combinations of GRAS chemicals and temperature".

- Greer G.G. and Dilts B.D., Factors affecting the susceptibility of meat borne pathogens and spoilage bacteria to organic acids.
  Food Research International 25: 355-362 (1992).

## 1. Toxicokinetics, Metabolism and Distribution

- (L)-lactic acid is a natural functional metabolite in mammal, as mammalian fuel.
  According to the lactate shuttle concept, L-lactate represents a major means of distributing carbohydrate potential energy for oxidation and gluconeogenesis. The concept of a "lactate shuttle" (Brooks, 1998) is that during hard exercise, as well as other conditions of accelerated glycolysis, glycolic flux in muscle involves L-lactate formation regardless of the state of oxygenation. The production rate of endogenous (L)-lactate in the resting human is about 1.3 mol (70 kg/bw).24 h-1 (= 117 g/day).

## 2. Acute oral toxicity

**LD50**

| | |
|---|---|
| Title | Acute oral LD50 study in rats |
| Species: | Charles River male/female rats |
| Report number | 410-1369 |
| Year | 1984 |
| GLP | Yes |
| Test substance & purity | L(+) lactic acid 80%, diluted with water. |
| Exposure / dosage | Oral dosage; 3,162 / 3,548 / 3,981 / 4,467 / 5,012 / 5,623 / 6,310 mg /kg bw |
| Exposure Time | One dosage |
| No. of Animals | 55 |
| Experimental design | Method: EPA OPP 81-1 |
| Observations | The following mortalities were observed during the main study testing:<br>dosage mg/kg: 3,162 3,548 3,981 4,467 5,012 5,623 6,310<br><br>males -- -- -- 1/5 3/5 4/5 5/5<br>females 1/5 2/5 5/5 5/5 5/5 5/5 5/5<br><br>All mortalities occurred after dosing on day 0 or in morning of day 1, except for one female dosed at 3,162 mg/kg that was found dead on the morning of day 2. The animals were sacraficed after 14-day observation.<br>Consistent body weight gains were observed on days 7 and 14 for all surviving study animals.<br>Lethargy, ataxia, prostration, irregular breathing, piloerection, squinting, lacrimatiobn, salvation, crusty eyes and muzzle, loose stools, damp or yellow/brown stained fur, and moribund were abnormal clinical signs observed as early as 0 – 1 hour after dosing and as late as day 2. No other abnormal clinical signs were observed during the study.<br><br>Abnormal necropsy findings were observed for all found dead animals and for the 4 surviving females dosed at 3,162 mg/kg.<br>Abnormalities observed during necropsy of found dead animals included: discolored lungs; firm texture of lungs; green foci on one lung; erosion of stomachs; dark, black, brown and/or fluid contents of stomachs; black and/or brown discolored stomachs; a distended stomach with white mucosa; mucosal sloughing, ulceration and hemorrhage of the stomachs; discolored livers; white foci on livers; pale capsular areas, superficial erosion, or mottled livers; a discolored diaphraghm; green-black or brown-black discolored kidneys; and red-brown exudate in the nasal and/or oral regions.<br>Mottled lungs were observed during necropsy of 3 surviving animales dosed at 3,162 mg/kg and thickened stomahcs were also observed during necropsy of 2 surviving animals of the same group. No other abnormalities were observed during necropsy of all study animals. |
| Calculation of LD50 | The oral LD50 value, the 95% confidence interval, the slope of the response curve, and correction factors for 0 and 100% observed responses were calculated for each sex using a method adapted from Litchfield and Wilcoxon.[1] Dose-reponse curves were prepared using the calculated LD50 data. |
| Conclusion | $LD_{50}$ between 3543 and 4936 mg/kg bw |
| Remark | |
| | |

[1] Lichtfield, J.T., Jr. and Wilcoxon, F., „A simplified method of evaluating Dose-effect experiments“, Journal of Pharmacology and Experimental Therapeutics, vol 96, 1949, pages 99-113.

**LD100**

| | |
|---|---|
| Title | LD 100 |
| Species: | 10 rats |
| Concentration: | Oral dosage; dose was daily increased: 0.25 ml till 4.5 ml lactic acid 50% |
| Report number | |
| Year | |
| GLP | |
| Test substance & purity | L(+) lactic acid 80% water |
| Exposure | |
| Exposure Time | |
| No. of Animals | |
| Experimental design | |
| Observations | 2 Rats died after dosing with 3 ml = 7500 mg/kg bw. The animals had a 15% reduction in bw in 1 week. A single administration of large doses did not result in changes in carbon dioxide content or pH of the blood, but a considerable decrease in the pH of the urine. |
| Conclusion | $LD_{100}$: = 11250 mg/kg bw |
| Remark | |
| | |

**Acute toxicity**

| | |
|---|---|
| Title | Acute oral toxicity study in rats |
| Species: | Rats, Charles River |
| Report number | 410-1353 |
| Year | 1983 |
| GLP | Yes |
| Test substance & purity | L(+) lactic acid 80%, diluted with water |
| Exposure | 5 mg / kg bodyweight |
| Exposure Time | One dosage |
| No. of Animals | 10, 5 male and 5 female |
| Experimental design | EPA OPP 81-1. The duration of the study was 14 days. The animals were fasted overnight. The following morning, body weights were recorded, doses were calculated and a measured volume of test article was delivered to each animal by gavage in a single dose. Diet was returend to each animal approx 4 hours after test article administration. Observations for mortality and abnormal clinical signs were done twice daily. Body weights were recorded prior to test article administration on day 0, on day 7 and prior to sacrifice on day 14. Also final body weight were recorded prior to necropsy for animals found dead. |
| Observations | Body weight gains were observed for 3 surviving males and a small weight loss was observed for the fourth surviving animal. Body weight loss was observed for all the animals that were found dead. Lethargy and salivation were observed for all animals and crusty muzzle was observed for 9 animals on the day of dosing and as late as day 9 for one female. Other abnormal clinical signs observed for some animals on the day of dosing and as late as day 2 included ataxia, prostration, irregular breathing, noisy breathing, squinting, lacrimation, crusty eyes, crusty nose and body cool to touch. Other abnormal clinical signs observed prior to death of one female on day 10 included yellow/brown stained fur in the perianal region, abdomen swollen, no stools, and few stools.<br>No other abnormal clinical signs were seen during the study. |
| Conclusion | Four males survived the 14-day duration of the study. One male and all females were found dead on the day of dosing (day 0), on day 1 or on day 10. |
| Remark | Test was done to establish clinical signs after single dose treatment |

## 3. Acute Inhalation Toxicity

**Acute Inhalation toxicity**

| | |
|---|---|
| Title | Acute Inhalation toxicity study |
| Species: | Rat Fischer 344 male/female |
| Concentration: | |
| Report number | I-7083.112 |
| Year | 1987 |
| GLP | Yes |
| Test substance & purity | |
| Exposure | Aerosol containing 7,94 mg/L (nose only) a second group was exposed to air alone. |
| Exposure Time | 4 hour(s) |
| No. of Animals | 10 |
| Experimental design | EPA OPP 81-3; The animals were observed for mortality and pharmacotoxic signs after exposure, at 1 and 3 hours following exposure and once daily after that for 14 days. Complete necropsis were performed on all animals on day 15 of the study. Histopathology was not performed |
| Observations | Animals were observed during exposure for signs of toxicity. Rapid breathing and eye tearing was observed in the treatment group. One and three hours after exposure, the treated and control groups had a hunched posture, red stained fur surrounding the eyes(tearing), ruffled fur, and appeared ungroomed with solid fur. Female rats exposed to the test substance appeared lethargic at one (2/5) and three hours (5/5). The two female rats that were lethargic afte 1 hour also had a rapid, shallow breathing and appeared to be gasping afte one and three hours. By 24 hours, most animals appeared normal and no unusual behavious or appearance was observed for the remainder of the test period. However, of the treated female rats 4/5 hd ruffled, ungroomed fur at 24 hours, and 3/5 had ruffled, ungroomed fur 2, 3 and 4 days after treatment. One female from the treatment group had hunched posture, rapid and shallow breathing, and slight tremors, but these signs were observed on day 5 post-treatment. One female from the treament group died on day 8 post-treatment. No gross lesions were observed at necropsy. |
| Conclusion | Based on these results, the LC50 for L(+)Lactic Acid is greater than 7,94 mg/L. |
| Remark | |

## 4. Acute Dermal Toxicity

- Type: Dermal LD50
  Species: rabbit
  Value: > 2000 mg/kg bw
  Method: OECD Guide-line 402 "Acute dermal Toxicity"

**Acute dermal toxicity**

| | |
|---|---|
| Title | Acute dermal toxicity study in rabbits |
| Species: | Rabbits, New Zeeland white, male and female |
| Report number | 410-1354 |
| Year | 1983 |
| GLP | Yes |
| Test substance & purity | L(+) lactic acid 80%, diluted with water |
| Exposure | 2 g/kg for 2 hours of exposure |
| Exposure Time | 2 g/kg for 2 hours of exposure |
| No. of Animals | 10 |
| Experimental design | EPA OPP 81-2; Approx 24 hours after clipping the skin were abraided sufficiently deep to penetrate the stratum corneum but not the dermis. After test substance application, the trunk of each animal was wrapped. After a 24 hour exposure period, each binder was removed and the test site of the animal was wiped to remove the remaing test article.<br>Animals were observed for mortality and abnormal clinical signs hourly after dosing on day 0. Observations for mortality and abnormal clinical signs were done twice daily thereafter during the duration fo the study (14 days). On day 14 all animals were rendered unconscious and were exsanguinated prior to gross necropsy. |
| Observations | All animals survived the 14-days duration of the study and gained body weight. No abnormal clinical signs were observed during the study. Severe erythema and severe oedema were observed at the test sites of all animals after removal on day 1. Erythema decreased in severity for 3 animals on day 12 or 14, and was not observed for one female on day 14.<br>Oedema decreased in severity for 8 animals and was not observed for one female on day 12 and for one male on day 14. Other dermal reactions observed at test sites included: blanching, necrosis, eschar formation, atonia, desquamation and denuded areas.<br>Other dermal reactions observed at test site included:<br>Blanching, necrosis, eschar formation, eschar peeled off, atonia, desquamation, fissures, denuded areas along abraised lines.<br>Brown, crusted and raised discolorations of the treated skin were observed during necropsy of 3 males and 3 females. Multiple depressions in the treated skin were observed during necropsy of one of the same males, of 2 other males , and of one other female. A dark red focus was observed on the lung of one male. No other abnormalities were observed during necropsy of all males and 4 females, and no abnormalities were observed during necropsy of one female. |
| Conclusion | Dermal LD50 >2000 mg/kg in rabbits |
| Remark | |
| | |

## 5. Skin Irritation

| | |
|---|---|
| Title | Acute dermal irritation/corrosion test with lactic acid (88%) in albino rabbits |
| Species: | Rabbit |
| Concentration: | 88 % |
| Report number | V 86.016 |
| Year | 1986 |
| GLP | Yes |
| Test substance & purity | L(+) lactic acid 88%, diluted with water |
| Exposure | Occlusive |
| Exposure Time | 4 hour(s) |
| No. of Animals | 12 |
| Experimental design | OECD Guide-line 404 "Acute Dermal Irritation/Corrosion"; One day prior to the experiment the hair was removed form the back and flanks of the animals. Six rabbits were treated on the intact skin and on the abraided skin. The abraisions were minor incisions of the stratum corneum, but not sufficient to disturb the underlying derma or to produce bleeding. An amount of 0,5 ml of the test material was brought onto the intact and the abraided skin under a surgical patch measuring 1 inch x 1 inch.<br>After an exposure period of 4 hours the patches and the material applied were removed and the resulting skin reactions were evaluated by the method of Draize et.al. (J. Pharmacol. Exptl. Therap. 82 (1944) 377-390). |
| Observations | After 4 hours the dermal effects generally oserved in all rabbits concisted of very slight to slight ischemic necrosis, moderate to severe haemorrhages and slight or moderate oedema. After 28 hours the dermal effects observed generally consisted of very slight to slight ischemic necrosis, moderate haemorrhages, slight or moderate incrustation and slight oedema.<br>During the course of the following two days ischemci necrosis, haemorrhages and oedema were no longer observed. The application sites generally became crater-shaped with a central sunken area which was moderately or severely encrusted, and a surrounding, raised border of non-necrotic skin showing well-defined erythema.<br>After 7 days this picture had hardly changed, apart from clearance of erythema. The central sunken areas of the application sites generally showed moderate to severe incrustation.<br>At the end of the observation period, after 3 weeks, soms signs of healing were observed at the edges of the encrusted skin areas which had been in contact with the tst material. In the new skin visible under the crust edges coming off from the treated skin, formation of scar tissue could be observed whereas hairgrowth was absent. There were no differences between reactions of the intact skin and those of abraided skin. This scar tissue formed already or to be formed is not considered a reversible skin alteration. |
| Conclusion | On the basis of the results obtained, it can be concluded that lactic acid (88%) is severely irritating to the skin of albino rabbits and that tlactic acid (88%) is corrosive to the skin of albino rabbits. |
| Remark | Other studies have shown that the skin of albino rabbit is not the appropriate animal model when addressing the effects of lactic acid on human skin. This result is therefore not used for the classification. |
| | |

| | |
|---|---|
| Title | Acute dermal irritation / corrosion study with PURAC BF S36 and PURAC BF S/30 in albino rabbits |
| Species: | New Zealand White albino rabbits |
| Concentration: | buffered lactic acid: BF S36 (38% l.a. + 38% sodium lactate, total 76% |

| | |
|---|---|
| | d.s.). BF S30 (60% l.a. + 20% sodium lactate, total 80% d.s.) |
| Report number | V 96.677 |
| Year | 1996 |
| GLP | Yes |
| Test substance & purity | buffered lactic acid: BF S36 (38% l.a. + 38% sodium lactate, total 76% d.s.). BF S30 (60% l.a. + 20% sodium lactate, total 80% d.s.) |
| Exposure | Occlusive |
| Exposure Time | 4 hours. |
| No. of Animals | 3 |
| Experimental design | Four days rpior to the start of the study, the hair was removed from the back and the flanks of the animals in a way to avoid abrasions. Each rabbit was treated simutanneously with the same compound.<br>An amount of 0,5 ml of each test substance was distributed over a pathc measuring 2.5 x 2.5 cm. The two patches were fixed to the application sites. Subsequently, the entire trunk fo the rabbit was wrapped with self-adhesive tape.<br>After a 4 hour exposure period, the test substance and patches were removed and the test sites were cleanded with moistened tissues.<br>After a hour the resulting skin reactions were evaluated by the method of Draize et al (J. Pharmacol. Exp. Ther. 82 (1944) 377-390). Further skin readings were made at approximately 24, 48 and 72 hours after treatment. |
| Observations | After 1-72 hours after treatment, no signs of skin irritation were observed in any of the three rabbits. |
| Conclusion | On the basis of the results obtained, it was concluded that, udner the conditions fo this study, PURAC BF36 and PURAC BF S30 are not irritating for the skin of rabbits after a 4 hour dermal contact period. |
| Remark | |

| | |
|---|---|
| Title | Primary dermal irritation study in rabbits |
| Species: | New Zealand White albino rabbits |
| Concentration: | 80% |
| Report number | 410-1355 |
| Year | 1983 |
| GLP | Yes |
| Test substance & purity | L(+) lactic acid 80%, diluted with water |
| Exposure | Occlusive |
| Exposure Time | 24 hours |
| No. of Animals | 6, 3 male and 3 female |
| Experimental design | Two test sites were prepared on each side of the spinal column in the thoracic region of each animal by closely clipping the hair.<br>Approximately 24 hours after clipping, 0.5 ml of the test article was applied to each 2.5 cm square surgical gauze patch. 2 Application sites on each animal were abraded with a needle to penetrate the stratum corneum but not the dermis. Four patches containg the test article were then applied to each animal and held in place with gauze wrapping.<br>After 24 hours exposure, the patches were removed. Each test site was gently wiped with gauze sponges moistened with water to remove the remainign test article.<br>The skin conditions of each test site was evaluated for erythema, edema and other lesions at 30 and 60 minutes after test article removal. After 30-60 minutes evaluations all animals were euthanized due to severity fo the dermal reactions observed.<br>EPA OPP 81-5 |
| Observations | - Severe erythema at 10 of the 12 abraded test sites (on 5 animals) and at 7 of the 12 intact test sites on 4 of the same animals. Moderate to severe erythema was seen at 2 remaining abraded and 5 remaining intact test sites.<br>- Severe edema at 11 of 12 intact test sites and at 11 of 12 abraded test |

| | |
|---|---|
| | sites. Slight edema at one abraded test site and one intact test site on one animal.<br>- Blanching at both abraded sites on each animal and at both intact sites at 5 animals.<br>- Yellow-brown color of skin at all sites on 3 animals, at both abraded sites on one animal and at one intact site and both abraded sites on a fifth animal.<br>- Red exudate at one intact site on one animal.<br>- Skin missing at all sites of one animal, at one intact site and both abraded sites on one animal, and at one intact site or one abraded site on 2 other animals.<br>No other dermal reactions were observed at the evaluations done at 30 to 60 minutes after test article removal. No abormal clinical sign were observed and no mortalities occurred prior to sacrifice after 30 – 60 minute evaluations. |
| Remark | Under the definition of CFR 49, 173.136, the product does not need to be classified |
| | |

| | |
|---|---|
| Title | Lactic acid Q88: A skin corrosivity test in guinea pigs |
| Species: | Guinea pigs, Dunkin Hartly strain |
| Concentration: | 88% |
| Report number | 235943 |
| Year | 1986 |
| GLP | Yes |
| Test substance & purity | L(+) lactic acid 88%, diluted with water |
| Exposure | Occlusive |
| Exposure Time | Up to 4 hours |
| No. of Animals | 6 |
| Experimental design | The animals were divided inot 2 groups of 3. The hair was clipped from both flanks 24 hours before patch application. Care was taken to avoid abrading of the skin.<br>In group 1, 0.5 ml of the test material was applied (at two sites) under 2 gauze patches each measuring 2.5 x 2.5 cm. The patches were applied to the intact skin and covered with Micropore tape. The whole trunk was then bound with Elastoplast elactic bandage to give a semi-occlusive covering.<br>Group 2 animals were treated in the same way except that the test material was applied under one patch.<br>For group 1 animals the first patch was removed 3 minutes after application and the skin was jently washed with water to remove any residual test material. Reactions were scored. One hour after application the second patch was removed, the skin washed and reactions scored.<br>The patch for group 2 animals was left in place for 4 hours before removal and scoring.<br>Skin reactions were scored for erythema and eschar formation and for oedema formation at patch removal, 1, 24, 48 and 72 hours after patch removal. |
| Observations | In group 1 ( 3 minutes and 1 hour exposure) no erythema, no eschar formation and no oedema formation was seen.<br>In group 2 ( 4 hours exposure) very slight erythema (barely perceptible) and very slight oedema formation (barely perceptible) was seen at patch removal and after one hour.<br>In group 2 no erythema, no eschar formation and no oedema formation was seen at 24, 48 and 72 hours after patch removal. |
| Conclusion | It is concluded from the test results that lactic acid 88% is not corrosive to guinea pig skin. It is also noted that irritation after 4 hours exposure was transient and limited to very mild erythema. |
| Remark | |
| | |

| | |
|---|---|
| Title | Acute dermal irritation/corrosion study with lactic acid 88% in pigs |
| Species: | Healthy male, young pigs from Large white (GY) x Dutch Landrace (NL) |
| Concentration: | 88% |
| Report number | V 87.405 |
| Year | 1987 |
| GLP | Yes |
| Test substance & purity | L(+) lactic acid 88%, diluted with water |
| Exposure | Dermal, 0,5 ml test material per application site, occlusive. |
| Exposure Time | Up to 4 hours |
| No. of Animals | 3 |
| Experimental design | The hair was removed from the animals. The test material was brought into contact with three small separate areas of intact skin. Each of the application sites was covered with occlusive patch measuring 1 inch x 1 inch.The first patch was removed after 3 minutes, the second after 1 hour and the third after 4 hours. Immediately after removing the patches the treated skin areas were treated with lukewarm water, and one hour later the resulting skin reactions were evaluated by the method of Draize et.al. (J. Pharmacol. Exp. Ther. 82 (1944) 377-390). Further readings were made after 1 day and after 2, 3, 7, 14 and 21 days. |
| Observations | No dermal irritaton responses related to treatment with lactic acid (88%) were observed at the test sites of any animal during the 21-day observation period, etiher after a 3 minute contact epriod, after 60 minutes contact period or after a 4-hour contact period.<br>After one day, superficial wounds were observed at the test site of pig no. 1 treated for 60 minutes and at the test site of pig no. 3 treated for 4 hours. In the same animals, similar injuries were also seen at the non-treated skin. After two days, the same test sites and non-treated skin areas in pigs 1 and 3 showed some slight small crusts. After three days these affects had cleared. These minor injuries were probably caused by shaving along the walls or the floor of the stable. |
| Conclusion | Under the conditions of this test it is concluded that lactic acid (88%) is not a primairy skin irritant to the pig skin. |
| Remark | TNO and other experts believe the pig to be a more appropriate and representative animal model than the albino rabbit, when addressing the effects of lactic acid on human skin. |
| | |

## 6. Eye Irritation

| | |
|---|---|
| Species | rabbit |
| Concentration: | 20 % L(+) lactic acid |
| Year | 1973 |
| GLP | yes |
| Test substance & purity | 20 % L(+) lactic acid in water |
| Experimental design | Journal Officiel de la Republique Francaise procedure; eyes were examined after 1 and 24 h and after 2, 3, 4, and 7 days with fluorescent staining. |
| Observations | In same study also 50% sodium lactate was tested, which is not irritating. Instilled at 20% and 10% provoked significant ocular irritation :Acute Ocular Irritation Index (AOII) was 39.50 resp. 31.17. Only for the 10% dilution these lesions were reversible, 7 days after instillation. |
| Conclusion | irritating |
| Remark | **From public literature, see references.** |
| | |

| | |
|---|---|
| Title | Chicken Enucleated eye test with three samples of lactic acid; an alternative to the Draize eye test. |
| Species: | Male or female chickens (ROSS, spring chickens) were used as eye donor. |
| Concentration: | |
| Report number | V96.157 |
| Year | 1996 |
| GLP | Yes |
| Test substance & purity | - Powder H60 (60% L(+) lactic acid and 40% calcium S lactate)<br>- HS88, 88% L(+) lactic acid in water.<br>- BF S36 L(+) lactic acid; L(+) lactic acid and sodium S lactae. |
| Exposure | Corneal |
| Exposure Time | 10 seconds |
| No. of Animals | 10 eyes |
| Experimental design | Within 2 hours after the kill the heads were carefully disseceted and placed in a superfusion apparatus. The eyes are checked for damages. Corneal thickness was measured. Per test sample three eyes were selected for testing whereas one eye was rinsed with iostonic saline only and served as a control of the experimental conditions.<br>At time t=0 0.03 ml / 0.03 g of the test sample was applied to the eye in such a way that the entire surface of the cornea was bathe with the test material. After a total exposure period of 10 seconds, the corneal surface was rinsed thoroughly with with 20 ml of isotonic saline of ambient temperature. The control and the test eyes were examined for changes in corneal thickness (swelling), corneal opacity and fluorescein retention at 0, 30, 75, 120, 180 and 240 minutes after treatment. |
| Observations | - <u>powder H60</u><br>After treatment the thickness of the cornea of the eyes gradually increased considerably; a maximum mean corneal swelling of 17% was obtained at 240 minutes after treatment. In addition, moderate corneal opacity and moderate fluorescein retention by damaged epithelial cells were observed in the eyes.<br>The categories defined for corneal swelling, corneal opacity and fluorescin retention were II, III and III<br><br>- <u>PURAC lactic acid HS88</u><br>After treatment severe to complete corneal opacity was observed in the three test eyes, which hampered the measurement of corneal thickness |

| | |
|---|---|
| | at the 30, 75 and 120 minutes after treatment. At 180 and 240 minutes after treatment corneal thickness could be measured again and at 240 minutes a maximum corneal swelling of 28% was determined. All three eyes showed severe fluorescein retention by damaged epithelial cells. The categories defined for corneal swelling, corneal opacity and fluorescin retention were III, IV and IV<br><br>- PURAC lactic acid bufered BF S36<br>After treament only a slight increase in cornela thickness was observed. A maximum corneal swelling of 6% was obtained 75 minutes after treatment. Very slight corneal opacity and slight fluorescein retention by damaged epithelial cells were observed in the test eyes. The categories defined for corneal swelling, corneal opacity and fluorescin retention were II, I and II<br><br>- Control eye<br>The control eye did not show any unusual effects. |
| Conclusion | - powder H60<br><br>Irritating to the eyes<br><br>- lactic acid HS88<br><br>Severely irritating to the eyes<br><br>- lactic acid bufered BF S36<br><br>Not irritating to the eye. |
| Remark | The measurement of the cornela swelling in this test guarantees a highly objective parameter, which enables the investigator to discriminate the damaging effects of the test material very precisely, this in contrast to the conventional rabbit test which uses subjetive gross measurement only. |
| | |

| | |
|---|---|
| Species: | other |
| Concentration: | 85 % |
| Experimental design | Test condition: in vitro using the Eytex Assay (Avon Products, Inc, 1995). Most of the formulations were tested undiluted. pH of formulations varies from 7.52 to 2.02. |
| Observations | Only formulation with pH 2.02 (face cream with 11.8% lactic acid 85%) was moderate severe irritant. The formulations with pH ≥ 5.3 were minimal irritant. |
| Conclusion | irritating |
| Remark | **From public literature, see references.** |
| | |

## 7. Sensitisation

| | |
|---|---|
| Title | Dermal sensitization study in guinea pigs (Buehler Test) |
| Species: | Female, Hartley guinea pigs |
| Concentration: | L(+) lactic acid 80%, diluted with water |
| Report number | 480-2750 |
| Year | 1986 |
| GLP | Yes |
| Test substance & purity | L(+) lactic acid 80%, diluted with water |
| Exposure | Dermal |
| Exposure Time | Two days for the range finding and 35 days for the main study. |
| No. of Animals | 22 (2 for range finding, 10 for study test group and 10 for study naïve group) |
| Experimental design | The hair on the back or left flank (induction) and/or flank (range finding and challenge) of each animal was closely clipped. A 0.5 m sample of the test article and 0.5 ml samples of 30, 10 or 5 % suspensions of the test article in dionized water were placed on separate patches and applied to a range finding animal. The 100% test article was selected for induction. |
| Observations | Preliminary range-finding trials revealed very slight erythema at the 100% concentration of the test article. No other dermal reactions were noted for the other concentrations (3, 10 and 30%). Therefor the 100% concentration of the test article was utilized in the main study testing for contact dermal sensitization potential.<br>No mortalities occurred and all animals gained body weight. The test article (100%) produced very slight erytheam at 3 sites and very slight edema at 1 site after the first induction. Erythema grades increased in severity after the second induction application. Due to the increase of severity of the reactions, the concentration of the test article was reduced to 30% and the induction site was changed to the left flank. Very slight erythema was noted after the fifth induction application. Grades ranging from very slight to severe erythema were noted from the seventh to the nineth indction applications.<br>After the challenge application the test article (100%) produced grade 4 erythema in up to six test animals and in up to eight control animals. These reactions were considered irritation reactions, not sensitization reactions. The reactgions seen in the control animals at the challenge were similar to the reactions seen for the test group animals and the test article was not considered to be a cotnact dermal sensitizer. |
| Conclusion | The reactions seen (very slight to moderate erythema, very slight to moderate oedema) were considered to be irritant reactions, not sensitive reactions. The test article was not considered to be a dermal sensitiser. |
| Remark | |
| | |

## 8. Repeated Dose Toxicity

| | |
|---|---|
| Type | Sub-chronic |
| Species: | rat |
| Concentration: | 4 ml lactic acid 10% on 20 g of meal |
| Exposure Time | Exposure period: 90 days |
| Experimental design | Route of administration: gavage<br>Control Group: yes, concurrent no treatment<br>Frequency of treatment: every day |

| | |
|---|---|
| Observations | No differences in appearance, gross observations at necropsy, or organ weights were observed between the test and control animals. Changes in blood carbon dioxide were slight. |
| Conclusion | |
| Remark | **From public literature, see references.** |
| | |

| | |
|---|---|
| Type | Sub-chronic |
| Species: | female Sprague-Dawley rat |
| Test substance & purity | formulation (face cream containing 0.25% of lactic acid 85%) |
| Exposure | dermal |
| Exposure Time | 13 weeks treatment daily, 5 days/week<br>Control Group: yes, concurrent no treatment |
| Experimental design | Doses: 886 mg/kg bw |
| Observations | No significant gross observations, with the exception of minimal skin irritation. Absolute brain weight and kidney-to-body weight ratios were increased for test animals. No lesions were observed at necropsy or at microscopic examination. |
| Conclusion | The formulation is safe in terms of cumulative toxicity. Based upon the exaggerated dose level used in this study for skin care products, dermal application is not likely to produce adverse effects under conditions of consumer use.LOAEL: 886 mg/kg |
| Remark | **From public literature, see references.** |
| | |

| | |
|---|---|
| Type | Sub-chronic |
| Species: | Fischer 344 male/female rat |
| Concentration: | experiment I: calcium lactate dissolved in drinking water (up to 5%).<br>experiment II: up to 30% calcium lactate in diet. |
| Test substance & purity | Calcium lactate as a salt of lactic acid. |
| Exposure | Oral; 5, 2.5, 1.25, 0.6, 0.3 % |
| Exposure Time | Exposure period: 13 weeks |
| Experimental design | Control Group: yes, concurrent no treatment |
| Observations | a <10 % decrease in body weight gain. all animals survived. some haematological and biochemical parameters changed, but no severe lesions were found in microscopic examination in the experiment with ca-lactate mixed in the diet, the amount of calcium in the urine was significantly increased. Nephrocalcinosis and degeneration in kidneys observed. Indications that Nephrocalcinosis was dependent on the low Ca/Phosphorus ratio of the synthetic diet. |
| Remark | Lactic acid tested as its Calcium salt. From this study the lactate part is relevant, should be separated from effects of the soluble Calcium intake. **From public literature, see references.** |
| | |

| | |
|---|---|
| Type: | Sub-chronic |
| Species: | Syrian hamster male/female |
| Exposure | Group 1 (control):Diet 1, contains 20% sucrose as carcinogenic diet; pure water to drink. Group 2: diet 1, mixed with 0.057 ml lactic acid 80%; pure water to drink. Group 3: same diet 1, but water containing 0.050% v/v lactic acid |
| Exposure Time | 14 weeks |
| Experimental design | daily ad libitum; animals of groups 2 & 3 ingest same amount lactic acid.<br>Post exposure period: sacrificed and autopsy; also oral cavity (caries incidence)<br>Control Group: yes, concurrent no treatment |

| | |
|---|---|
| Observations | three groups same growth and health. No significant differences were found in the incidence or extent of carious lesions among the three groups. |
| Conclusion | dietary lactic acid did not play any important role in development or progress of dental caries. |
| Remark | pH of diet 1 is 5.55, of diet 2 is 5.12. pH of pure water is 6.8 and of water + lactic acid is 3.1 .<br>**From public literature, see references.** |
| | |

## 9. Genetic Toxicity 'in Vitro'

| | |
|---|---|
| Type: | Ames test |
| Species: | |
| Concentration: | 0.5, 1.0, and 2.0 microliter lactic acid/plate |
| Report number | |
| Year | |
| GLP | |
| Test substance & purity | |
| Exposure | |
| Exposure Time | |
| No. of Animals | |
| Experimental design | Salmonella/microsome test (Ames test) and chromosomal aberration test in vitro<br>reverse mutation assays, and Chinese hamster fibroblast cell line<br>S. typhimurium strains TA97, TA98, TA100, TA104<br>Metabolic activation: with and without |
| Observations | negative |
| Conclusion | |
| Remark | **From public literature, see references.** |
| | |

| | |
|---|---|
| Type: | Chinese hamster ovary K1 cells, chromosomal aberration tests, and the pH relationship of the medium and clastogenic activity was examined. |
| Concentration: | 8-35 mM |
| Experimental design | Cells were maintained in Ham's F12 medium, supplemented with 10% foetal calf serum.<br>Cytotoxic Concentration: 14-35 mM, when pH was <= 5.8<br>Metabolic activation: with and without |
| Observations | When the culture medium was first acidified by the lactic acid dose and then neutralised to pH 6.4 or when medium is containing 30 mM HEPES as buffer, lactic acid was non-clastogenic.<br>Pseudo-positive reactions are seen as a result of non-physiological low pH. |
| Conclusion | lactic acid was non-clastogenic |
| Remark | **From public literature, see references.** |
| | |

| | |
|---|---|
| Type | review on several mutagenicity studies with lactic acid and some lactates. |
| Concentration: | various |
| Experimental design | various<br>Metabolic activation: with and without |
| Conclusion | negative |
| Remark | the result of 11 studies is reviewed<br>**From public literature, see references.** |

## 10. Genetic Toxicity ' in Vivo'

Due to the natural nature of L(+) lactic acid and the relative low contribution of "outside L(+) lactic acid" to the human metabolism, in vivo genotoxicity studies will not be required.

- ***Carcinogenicity***

| | |
|---|---|
| Title | |
| Species: | Fischer 344 male/female rat |
| Concentration: | 2.5 or 5 % Calcium lactate in the drinking water. Mean total Calcium lactate intake for males was 329.4 g, resp. 625.4 g; for females 237.7 g, resp. 412.1 g. |
| GLP | yes |
| Test substance & purity | The Calcium salt of lactic acid was tested |
| Exposure | drinking water daily, ad lib |
| Exposure Time | 2 years |
| Experimental design | Control Group: yes, concurrent no treatment |
| Observations | Autopsy on rats that died during study and those killed at the end. Examination macro-and microscopically for presence of non-neoplastic and neoplastic lesions |
| Conclusion | Negative |
| Remark | Lactic acid tested as its Calcium salt. From this study the lactate intake is relevant, should be separated from the Calcium effects of a soluble Calcium salt. **From public literature, see references.** |
| | |

| | |
|---|---|
| Species: | female rabbit |
| Concentration: | 0.1-0.2 g/kg bw (5 months), and 0.1-0.7 g/kg bw (13 months) |
| Exposure | drinking water twice daily |
| Exposure Time | 5 or 13 months |
| Observations | No tumors were reported after 5 or 16 months. Further details not provided. |
| Conclusion | negative |
| Remark | **From public literature, see references.** |
| | |

## 11. Toxicity to Fertility

The nature of the compound (part of human metabolism) does make toxicity studies to fertility not necessary

## 12. Developmental Toxicity/Teratogenicity

| | |
|---|---|
| Species: | female CD-1 mouse |
| Exposure Time | gestational days 6-15<br>Duration of test: 10 days |
| Experimental design | Administration: gavage<br>Frequency of treatment: daily<br>Doses: 570 mg/kg bw/day<br>Control Group:yes, concurrent no treatment |
| Observations | Lactic acid was neither maternotoxic nor embryofetotoxic when given orally to mice at 570 mg/kg bw/day on gestation days 6-15. |
| Conclusion | NOAEL Maternal Toxicity: >= 570 mg/kg bw<br>NOAEL Teratogenicity: >= 570 ml/kg bw |
| Remark | **From public literature, see references.** |
| | |

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  1983

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- ***Toxicity to Fertility***

- ***Developmental Toxicity/Teratogenicity***

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# HPV Data Set

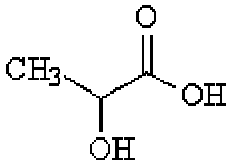
# LACTIC ACID

# CAS # 50-21-5

# Dossier number 50215

# Substance information

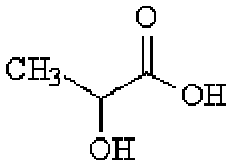
**CAS No.** 50-21-57
**EINECS Name**: lactic acid
**EC No.** 200-018-0
**TSCA Name**: Propanoic acid, 2-hydroxy-, (2S)-
**Molecular Formula** C3H6O3



**IUPAC Name:** LACTIC ACID
**Mol. Weight:** 90
**Physical status:** liquid or white crystals
**Colour:** colourless to slightly yellow
**Odour:** nearly odourless

**Most of the lactic acid nowadays is supplied in the L(+) form, the natural form.**
**PURAC only manufacturers the L(+) form.**
**Substance information for the L(+) lactic acid is as follows:**

**CAS No.** 79-33-4
**EINECS Name**: L(+) lactic acid
**EC No.** 201-196-2
**TSCA Name**: Propanoic acid, 2-hydroxy-, (2S)-
**Molecular Formula** C3H6O3



**IUPAC Name:** L(+) LACTIC ACID
**Mol. Weight:** 90
**Physical status:** liquid or white crystals
**Colour:** colourless to slightly yellow
**Odour:** nearly odourless

**This HPV data file typically contains information about L(+) lactic acid.**

# Chapter 1 Physico-chemical Data

- ***Melting Point***

| | |
|---|---|
| Value: | ≤ 54 degree C |
| Decomposition: | no at < 110 degree C |
| Sublimation: | no |
| Method: | other |
| GLP: | no data |
| Test substance: | 100% crystalline pure product used |

- ***Melting Point of the anhydrous form of the racemic mixture***

Kraft and Dyes found for the racemic lactic acid the melting point 18°C., Borsook, Huffman and Liu reported 16.8°C and more recently Lockwood, Yoder and Zienty reported values of up to 28-33°C. When one of the optically active isomers is present in excess, the melting point is higher; this indicates that racemic lactic acid forms a eutectic mixture.

Ref: ***Lactic Acid: properties and chemistry of lactic acid and derivatives by C.H. Holten, 1971, page 21***

- **Boiling Point / Vapour pressure**

| | |
|---|---|
| Boiling point: | ca. 258 degree C at 1000 hPa |
| Vapour pressure: | ca. .0041 hPa at 20 degree C |
| Decomposition: | yes |
| Method: | other |
| GLP: | no data |
| Remark: | solutions can polymerise on boiling |

It is not possible to determine this value very accurate, because lactic acid tends to polymerise by a polycondensation reaction.
The boiling point of lactic acid at difference vapour pressures according to

- Aspen Database
- PURAC In-house Pure compound database

**Table** Boiling points of lactic acid at different pressures (Aspen Database)

| Pressure (mbar) | Boiling temperature (°C) |
|---|---|
| 1 | 79.6 |
| 5 | 101.9 |

| | |
|---|---|
| 10 | 112.8 |
| 50 | 141.8 |
| 100 | 156.2 |
| 200 | 172.1 |
| 400 | 189.8 |
| 600 | 201.0 |
| 800 | 209.4 |
| 1013 | 216.6 |

The vapour pressures of lactic acid as a function of the boiling temperature according to the Aspen formula (PLXANT).

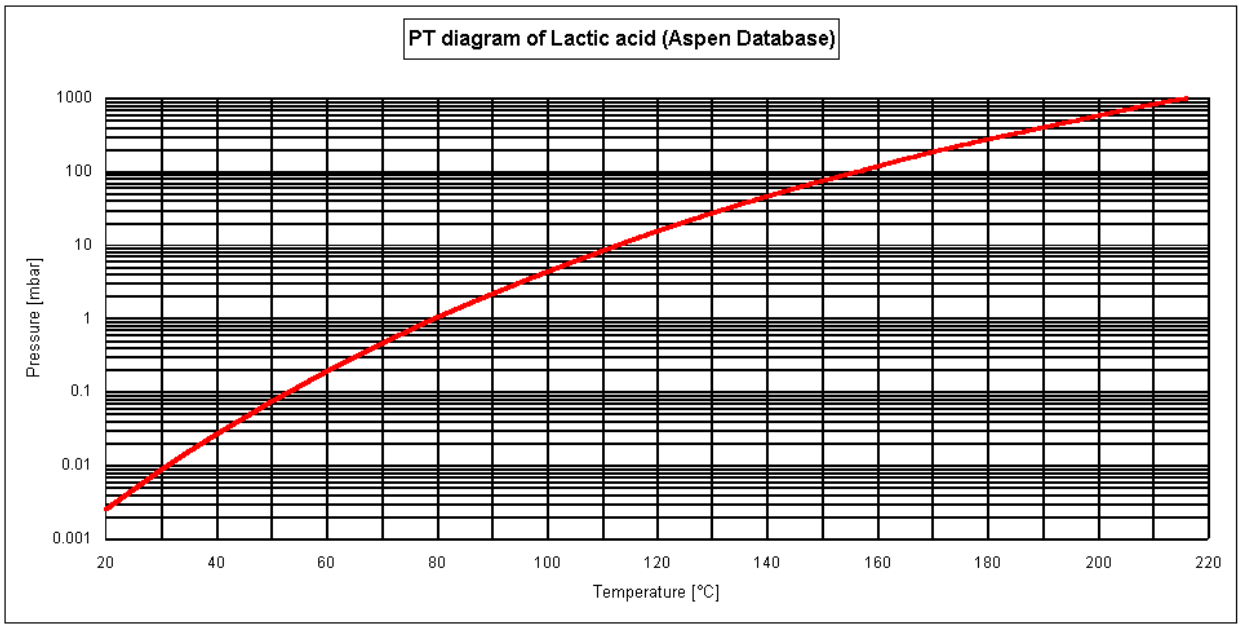
$$PLXANT = e^{C1+\frac{C2}{C3+T}+C4*T+C5*\ln T+C6*T^{C7}}$$

PLXANT : Vapour pressure ($N/m^2$ or Pa)

C1, C2 C3, C4, C5, C6, C7 : Regression coefficients for chemical compound

T : Boiling temperature (K)

**Figure** Vapour Pressure diagram




The regression coefficients are:

C1 = 225.19 C5 = -28.816

C2 = -18757 C6 = 0.000012998

C3 = 0 C7 = 2

C4 = 0

G.P.v.Lieshout measured the vapour pressure of lactic acid between 0 and 180 °C.

**Table** Boiling points of lactic acid at different pressures

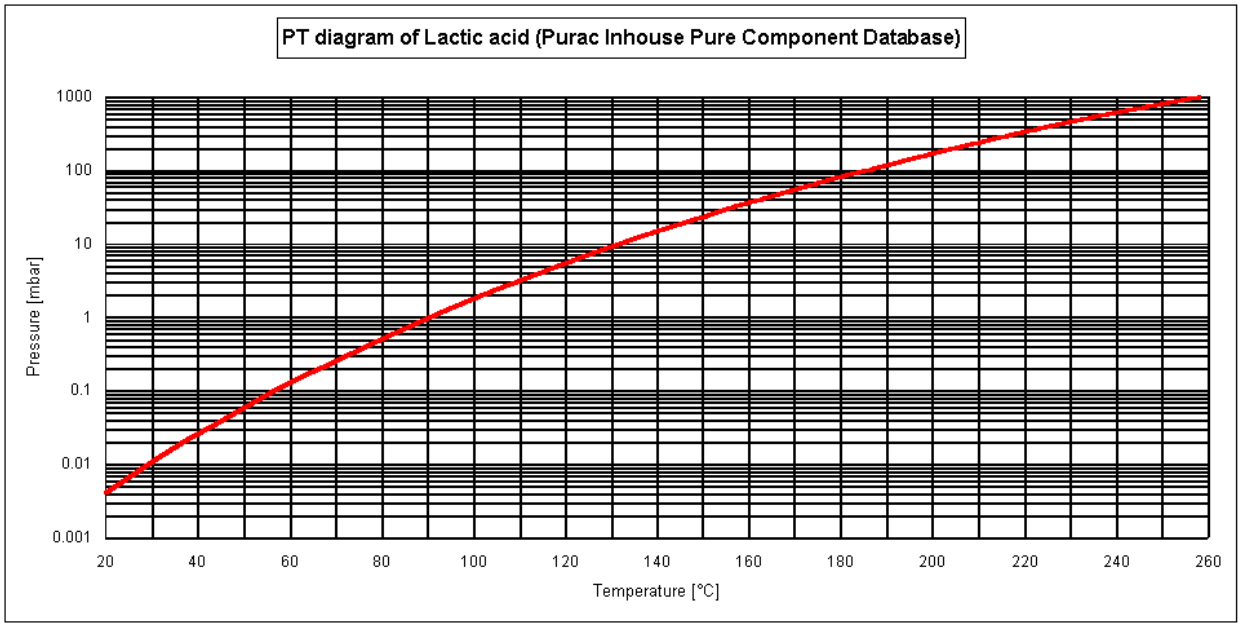
| Temperature (°C) | Vapour Pressure (mbar) |
|---|---|
| 0 | 0.0005 |
| 10 | 0.0015 |
| 20 | 0.0041 |
| 30 | 0.0107 |
| 40 | 0.0258 |
| 50 | 0.0588 |
| 60 | 0.1273 |
| 70 | 0.2629 |
| 80 | 0.5198 |
| 90 | 0.9877 |
| 100 | 1.8093 |
| 110 | 3.2042 |
| 120 | 5.5004 |
| 130 | 9.1736 |
| 140 | 14.8959 |
| 150 | 23.5938 |
| 160 | 36.5163 |
| 170 | 55.3114 |
| 180 | 82.1111 |

The vapour pressures of lactic acid as a function of the boiling temperature were fitted with the Aspen formula (PLXANT). The estimated atmospheric boiling point is about 258 °C.

$$PLXANT = e^{C1+\frac{C2}{C3+T}+C4*T+C5*\ln T+C6*T^{C7}}$$

PLXANT : Vapour pressure ($N/m^2$ or Pa)

C1, C2 C3, C4, C5, C6, C7 : Regression coefficients for chemical compound



: Boiling temperature (K)

Figure: Vapour Pressure diagram
The regression coefficients are:

| | |
|---|---|
| C1 = 32.4649 | C5 = -0.376037 |
| C2 = -8835.5 | C6 = 0 |
| C3 = 0 | C7 = 0 |
| C4 = -0.003654 | |

- **Density**

Type: density
Value: >= 1.2255 g/cm³ at 20 degree C
Method: other
GLP: yes
Test substance: Lactic acid 80%

Holten describes the liquid density of lactic acid solutions in water.
**Table** Liquid Densities of aqueous lactic acid solutions at different temperatures.

| Concentration (wt%) | Temperature (°C) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 10 | 20 | 30 | 40 | 50 | 60 | 70 | 80 | 90 | 100 |
| 0 | 1.0004 | 0.9997 | 0.9979 | 0.9953 | 0.9919 | 0.9878 | 0.9831 | 0.9778 | 0.9720 | 0.9658 | 0.9591 |
| 10 | 1.0269 | 1.0251 | 1.0223 | 1.0188 | 1.0145 | 1.0097 | 1.0042 | 0.9983 | 0.9919 | 0.9851 | 0.9779 |
| 20 | 1.0535 | 1.0505 | 1.0468 | 1.0424 | 1.0373 | 1.0317 | 1.0256 | 1.0190 | 1.0120 | 1.0047 | 0.9970 |
| 30 | 1.0802 | 1.0762 | 1.0715 | 1.0662 | 1.0604 | 1.0540 | 1.0472 | 1.0400 | 1.0325 | 1.0246 | 1.0164 |
| 40 | 1.1069 | 1.1019 | 1.0964 | 1.0902 | 1.0836 | 1.0766 | 1.0692 | 1.0614 | 1.0533 | 1.0449 | 1.0363 |
| 50 | 1.1333 | 1.1275 | 1.1211 | 1.1142 | 1.1069 | 1.0992 | 1.0912 | 1.0829 | 1.0743 | 1.0655 | 1.0564 |
| 60 | 1.1591 | 1.1524 | 1.1453 | 1.1377 | 1.1299 | 1.1216 | 1.1131 | 1.1043 | 1.0953 | 1.0861 | 1.0766 |
| 70 | 1.1835 | 1.1762 | 1.1685 | 1.1604 | 1.1521 | 1.1434 | 1.1345 | 1.1254 | 1.1160 | 1.1065 | 1.0967 |
| 80 | 1.2061 | 1.1983 | 1.1902 | 1.1818 | 1.1731 | 1.1642 | 1.1550 | 1.1456 | 1.1360 | 1.1263 | 1.1163 |
| 90 | 1.2263 | 1.2183 | 1.2100 | 1.2014 | 1.1925 | 1.1834 | 1.1741 | 1.1646 | 1.1550 | 1.1451 | 1.1351 |
| 95 | 1.2354 | 1.2274 | 1.2190 | 1.2104 | 1.2015 | 1.1924 | 1.1831 | 1.1736 | 1.1639 | 1.1541 | 1.1441 |
| 100 | 1.2438 | 1.2357 | 1.2274 | 1.2188 | 1.2099 | 1.2008 | 1.1916 | 1.1821 | 1.1725 | 1.1627 | 1.1527 |

**Liquid Density of <u>pure lactic acid</u>**

The liquid density of pure lactic acid at difference temperatures according to:

- Aspen Database
- PURAC In-house Pure Component Database

**Table** Densities of lactic acid at different temperatures (ASPEN Database)

| Temperature (°C) | Density (g/ml) |
|---|---|
| 20 | 1.2255 |
| 30 | 1.2163 |
| 40 | 1.2069 |
| 50 | 1.1974 |
| 60 | 1.1878 |
| 70 | 1.1780 |
| 80 | 1.1681 |
| 90 | 1.1580 |
| 100 | 1.1478 |
| 110 | 1.1374 |
| 120 | 1.1268 |
| 130 | 1.1160 |
| 140 | 1.1051 |
| 150 | 1.0939 |
| 160 | 1.0825 |

The liquid densities of lactic acid as a function of the temperature according to the Aspen equation for liquid density (DNLDIP):

$$DNLDIP = \frac{C1}{C2^{1+(1-\frac{T}{C3})^{C4}}}$$

DNLDIP : Liquid density (kmole/$m^3$)
C1, C2 C4 : Regression coefficients for chemical compound
C3 : Critical temperature (K)
T : Temperature (K)
The regression coefficients are:
C1 = 0.9798 C3 = 675
C2 = 0.24593 C4 = 0.2333

**Liquid density diagram**

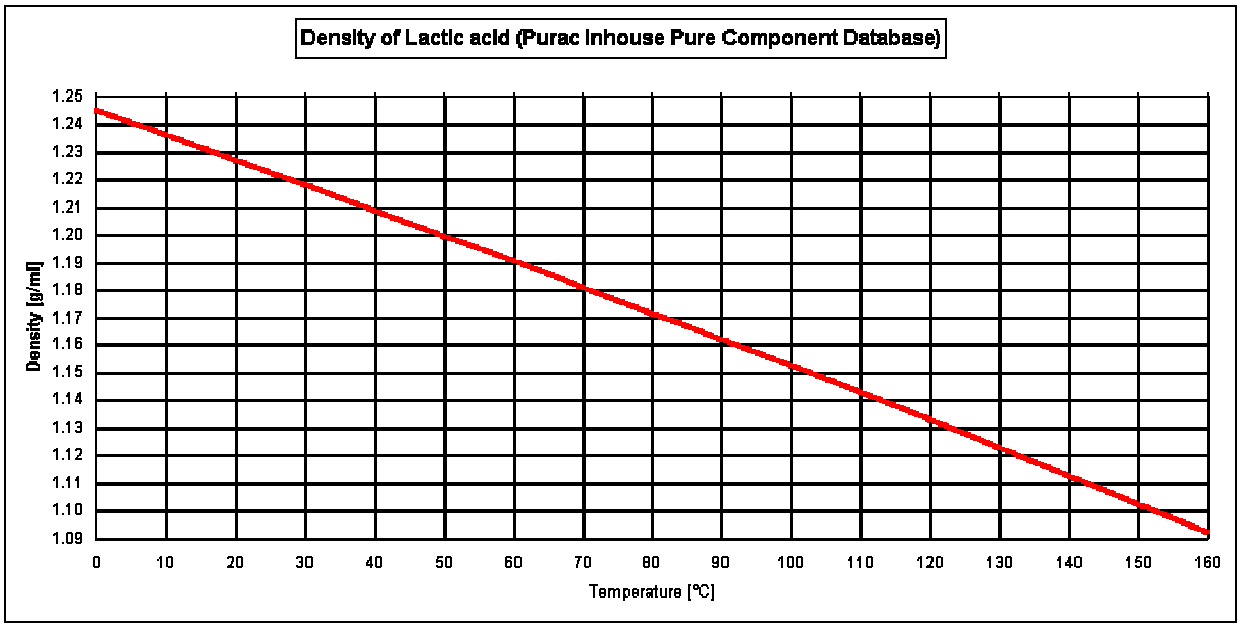



**Table** Densities of lactic acid at different temperatures (Purac In-house Pure Component Database)

| Temperature (°C) | Density (g/ml) |
|---|---|
| 0 | 1.2453 |
| 10 | 1.2364 |
| 20 | 1.2274 |
| 30 | 1.2183 |
| 40 | 1.2092 |
| 50 | 1.2000 |
| 60 | 1.1907 |
| 70 | 1.1813 |
| 80 | 1.1719 |
| 90 | 1.1623 |
| 100 | 1.1527 |
| 110 | 1.1429 |
| 120 | 1.1330 |
| 130 | 1.1231 |
| 140 | 1.1130 |
| 150 | 1.1028 |
| 160 | 1.0924 |

The liquid densities of lactic acid as a function of the temperature according to the Aspen equation for liquid density (DNLDIP):

$$DNLDIP = \frac{C1}{C2^{1+(1-\frac{T}{C3})^{C4}}}$$

DNLDIP : Liquid density (kmole/$m^3$)

C1, C2 C4 : Regression coefficients for chemical compound
C3 : Critical temperature (K)
T : Temperature (K)
The regression coefficients are:
C1 = 1.61248 C3 = 729.9
C2 = 0.314479 C4 = 0.328317

- ***Partition Coefficient***

Partition Coeff.: octanol-water
log Pow: ca. -.62 at 20 degree C
Method: OECD Guide-line 117 "Partition Coefficient (n-octanol/water), HPLC Method"
Year: 1987
GLP: yes

- ***Solubility in water***

Value: ca. 100 vol% at 25 degree C
pH value: ca. 1.2
pKa: 3.68 at 25 degree C
Descr.: of very high solubility
Method: other
GLP: no data
Deg. product: not measured
Stable: yes
Remark: completely soluble at 25 degrees C

J.v.Krieken determined the phase diagram of lactic acid/ water. Due to practical problems the diagram wasn't completed, but the missing part was extrapolated.

**Table** Solubility of monomeric lactic acid in water

| Temperature (°C) | Lactic acid (wt%) |
|---|---|
| -20 | 65.9 |
| -15 | 68.8 |
| -10 | 71.5 |
| -5 | 74.2 |
| 0 | 76.7 |
| 5 | 78.6 |
| 10 | 81.3 |
| 15 | 83.5 |
| 20 | 86.1 |
| 25 | 87.6 |
| 35 | 92.7 |
| 40 | 95.1 |

**Table** Freezing point data of monomeric lactic acid in water

| Temperature (°C) | Lactic acid (wt%) |
|---|---|

| | |
|---|---|
| 0 | 0 |
| -5 | 18.6 |
| -10 | 32.5 |
| -15 | 41.9 |
| -21 | 51.7 |

The eutectic point of lactic acid/water was calculated by extrapolation and is ca. 61.7 wt% lactic acid and -27.1 °C.

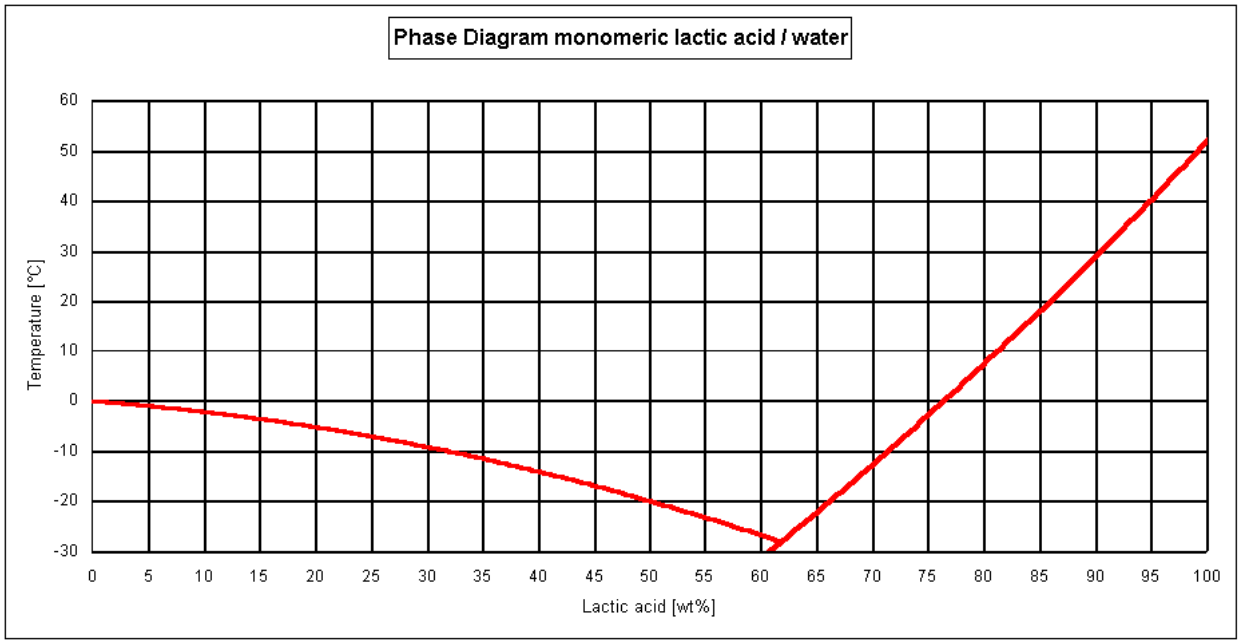



**Figure** Phase Diagram

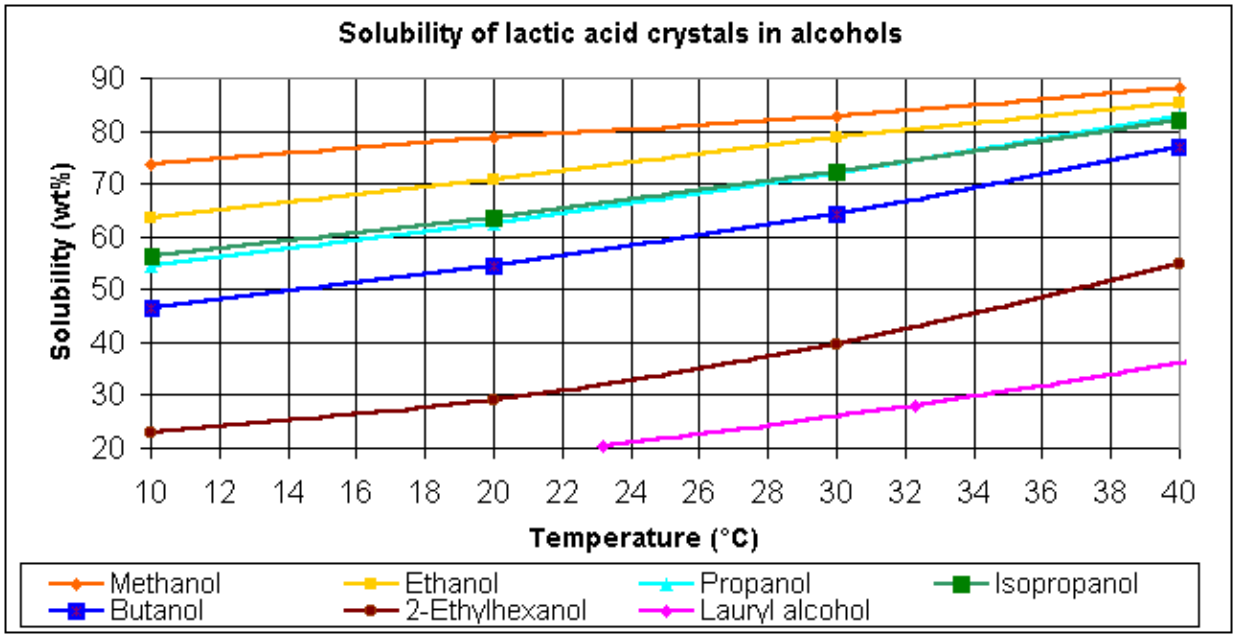
### Solubility of lactic acid in other solvents

J.v. Krieken investigated the solubility of pure lactic acid in a range of solvents by stirring the specific solvent with an excess of (S)-lactic acid crystals at a specific temperature. After separation of the solids from the liquid, the clear liquid was analysed on free acidity.

**Table** solubility of lactic acid in a range of solvents

| Solvent | Solubility (wt%) of solution | | | |
|---|---|---|---|---|
| | 10°C | 20°C | 30°C | 40°C |
| Methanol | 73.8 | 78.6 | 82.8 | 88.1 |
| Ethanol | 63.6 | 70.9 | 78.7 | 85.2 |
| 1-Propanol | 54.5 | 62.4 | 71.7 | 82.7 |
| 2-Propanol | 56.1 | 63.4 | 72.2 | 82.2 |
| 1-Butanol | 46.3 | 54.5 | 64.3 | 77.1 |
| 2-Ethyl-1-hexanol | 22.9 | 29.0 | 39.6 | 54.9 |
| Cyclohexane | | | | 0.04 |
| Hexane | < 0.01 | < 0.01 | < 0.01 | 0.02 |

| | | | | |
|---|---|---|---|---|
| Toluene | 0.06 | 0.11 | 0.24 | 0.50 |
| Ethyl lactate | 37.0 | 45.9 | 57.2 | 72.8 |
| Butyl lactate | 27.7 | 35.7 | 47.3 | 64.8 |
| 2-Ethylhexyl lactate | 15.5 | 20.9 | 30.9 | 46.7 |
| Ethyl acetate | 27.1 | 39.9 | 56.2 | 72.9 |
| Diethyl ether | 23.8 | 38.7 | 59.8 | |
| Diisopropyl ether | 6.4 | 9.2 | 15.9 | 44.4 |
| Tetrahydrofuran | 58.4 | 65.1 | 72.4 | 82.9 |
| Dichloromethane | 0.59 | 1.02 | 2.7 | |
| Chloroform | 0.31 | 0.67 | 1.67 | 59.7 |
| 2-Butanone | 40.7 | 52.9 | 67.3 | 81.6 |
| Acetone | 53.4 | 61.4 | 71.5 | 82.9 |




- ***Viscosity***

Test type: other: ASPEN database ; PURAC internal.
Value: ca. 53.52 mPa s (dynamic) at 20 degree C.

The liquid viscosity of lactic acid at difference temperatures according to.

- Aspen Database.
- PURAC In-house Pure Component Database.

The liquid viscosity of aqueous lactic acid solutions in water.

- 0 - 88 wt% of Lactic Acid.
- 90 - 110 wt% Lactic Acid.

**Table** Viscosity of lactic acid at different temperatures (Aspen Database).

| Temperature (°C) | Viscosity (cP) |
|---|---|
| 20 | 53.52 |
| 30 | 33.26 |
| 40 | 21.29 |
| 50 | 14.01 |
| 60 | 9.44 |
| 70 | 6.51 |
| 80 | 4.59 |
| 90 | 3.29 |
| 100 | 2.40 |
| 110 | 1.78 |
| 120 | 1.34 |
| 130 | 1.03 |
| 140 | 0.79 |
| 150 | 0.62 |
| 160 | 0.49 |

The liquid viscosity of lactic acid as a function of the temperature according to the Aspen equation for liquid viscosity (MULDIP):

$$MULDIP = e^{C1+\frac{C2}{T}+C3*\ln T+C4*T^{C5}}$$

MULDIP : Liquid viscosity ($N*s/m^2$ or Pa.s)
C1, C2 C3, C4, C5 : Regression coefficients for chemical compound
T : Temperature (K)
The regression coefficients are:
C1 = -14.403
C2 = 4097.9
C3 = -0.4407
C4 = 0
C5 = 0

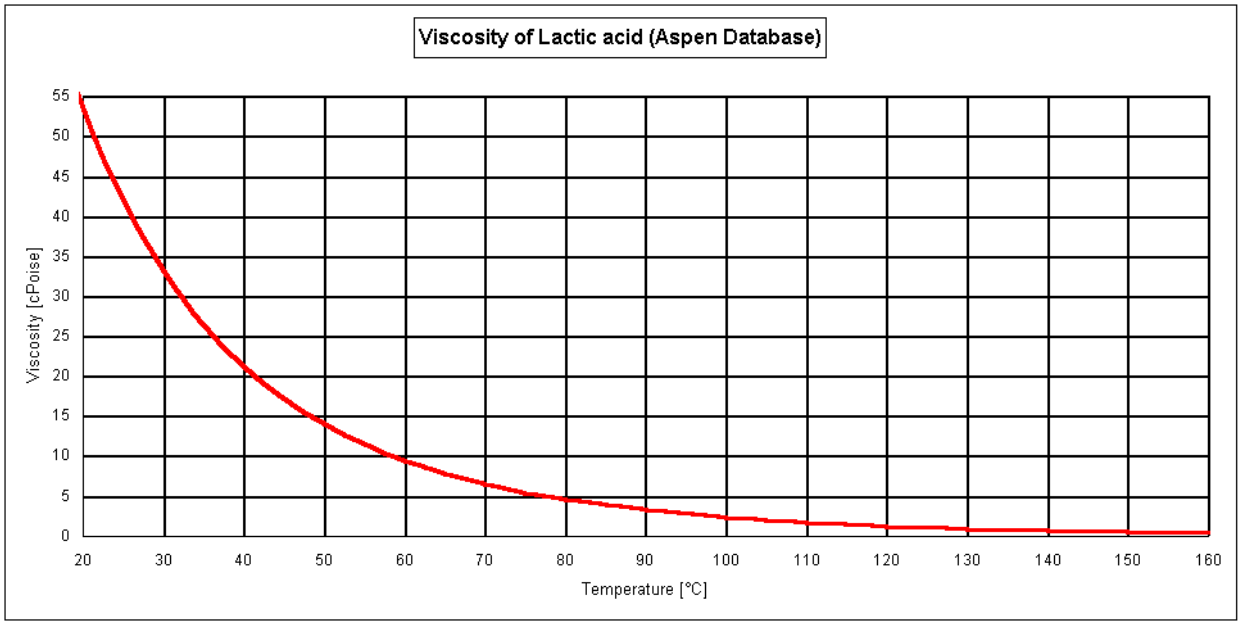


**Figure**: Liquid Viscosity diagram

Table 12 Viscosity of lactic acid at different temperatures (Purac In-house Pure Component Database) [2]

| Temperature (°C) | Viscosity (cP) |
|---|---|
| 20 | 1342.33 |
| 30 | 579.14 |
| 40 | 280.59 |
| 50 | 150.73 |
| 60 | 88.80 |
| 70 | 56.81 |
| 80 | 39.14 |
| 90 | 28.83 |
| 100 | 22.54 |

The liquid viscosity of lactic acid as a function of the temperature according to the Aspen equation for liquid viscosity (MULDIP):

MULDIP: Liquid viscosity ($N*s/m^2$ or Pa.s)
C1, C2, C3, C4, C5: Regression coefficients for chemical compound
T: Temperature (K)

The regression coefficients are:

C1: 421.094
C2: 25091.4
C3: 59.1119
C4: 0
C5: 0

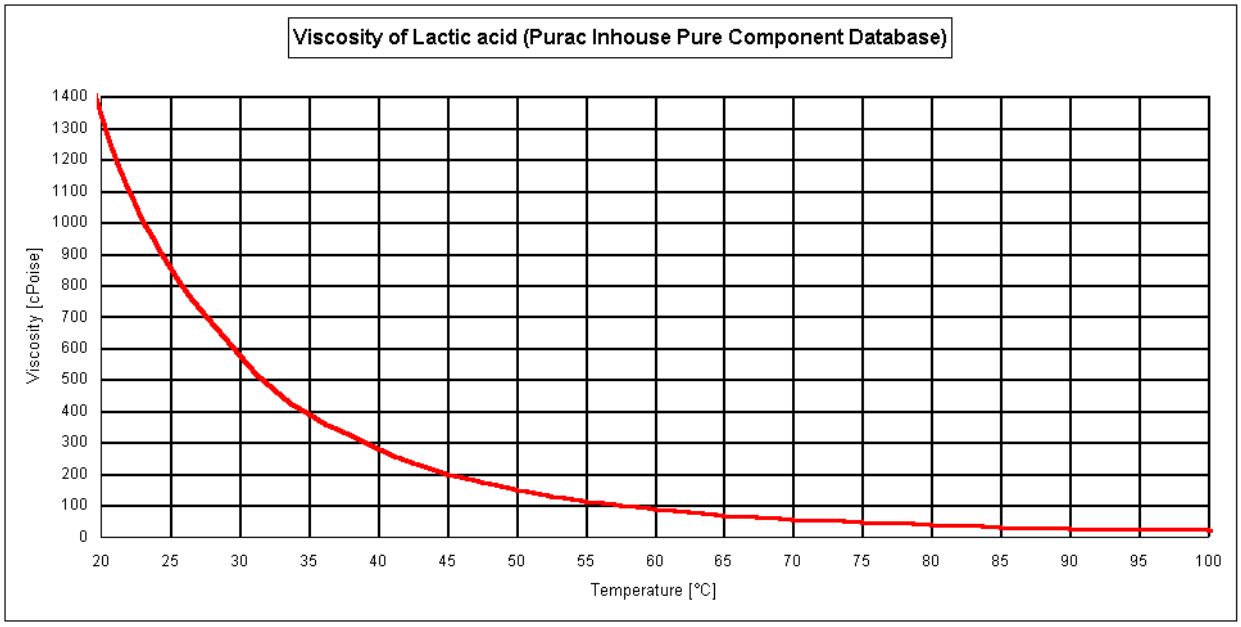



**Figure**: Liquid Viscosity diagram

## *References Chapter 1: Physico chemical data*


- National Chemical Inventories
  Coden NCINF5
  ISSN: 1089-6279

- Handbook of Chemistry and Physics
  WEAST
  66th Edition

- ASPEN database

- PURAC internal databank

- ***Photodegradation***

The photochemical oxidisation of lactic acid is discussed in "Lactic acid properties and chemistry of lactic acid and derivatives by C.H. Holten (1971)". The first observation that lactic acid is photosensitive was made in 1910 by Berthelot and Gaudechon, who irradiated calcium lactate and ethyl lactate with ultraviolet rays. They observed decomposition with the formation of gas containing carbon monoxide, carbon dioxide, hydrogen and methane.

Recognized method, i.e. OECD: Modelling conducted, no guideline studies used.

Method: Estimated Programs Interface (EPIWIN V3.05, Atmospheric Oxidation Program v 1.90). Model executed in October 2002.

Results / observations: The AOP component of EPIWIN was used to calculate the rate of photodegradation for L(+) lactic acid.

SMILES : O=C(O)C(O)C
CHEM : Propanoic acid, 2-hydroxy-, (S)-
MOL FOR: C3 H6 O3
MOL WT : 90.08

---------- SUMMARY (AOP v1.90): HYDROXYL RADICALS ----------

| | | |
|---|---|---|
| Hydrogen Abstraction | = | 5.2598 E-12 cm3/molecule-sec |
| Reaction with N, S and –OH | = | 0.6600 E-12 cm3/molecule-sec |
| Addition to Triple Bonds | = | 0.0000 E-12 cm3/molecule-sec |
| Addition to Olefinic Bonds | = | 0.0000 E-12 cm3/molecule-sec |
| Addition to Aromatic Rings | = | 0.0000 E-12 cm3/molecule-sec |
| Addition to Fused Rings | = | 0.0000 E-12 cm3/molecule-sec |
| OVERALL OH Rate Constant | = | 5.9198 E-12 cm3/molecule-sec |
| HALF-LIFE | = | 1.807 Days (12-hr day; 1.5E6 OH/cm3) |
| HALF-LIFE | = | 21.682 Hrs |

---------- SUMMARY (AOP v1.90): OZONE REACTION ----------

****** NO OZONE REACTION ESTIMATION ******
(ONLY Olefins and Acetylenes are Estimated)

Experimental Database: NO Structure Matches

- ***Stability in Water***

Lactic Acid (88% and 60% aqueous solutions) were investigated.
"The kinetics of degradation of Lactic Acid was done at elevated temperature, since the decomposition rates of lactic acid, (..) were too slow to obtain kinetic data within

reasonable time. At the condition studied (25, 40, 80 and 120 degrees centigrade) the decompositions of these compounds followed apparent first order kinetics because the mean correlation coefficient was above 0.980.
Lactic acid was very stable in aqueous solutions at 80 degrees centigrade (less than 30% decomposition after 175 at 80 degrees) and degradation was not different when combined with the various excipients tested. The shelf lives determined for lactic acid ranged from 79 years when combined with isopropyl palmitate to 98 years when combined with sorbic acid.

- ***Transport between Environmental Compartments***

Type: other: see free texts RM.
Remark: Lactic acid is not volatile and it has a high biodegradation rate. Therefor transport between compartments is no issue for this compound.

No experimental data are available on fugacity. However, calculation using the sotware program EPI Suite version 3.1 gave the following information which supports the statement given above:

| Level III Fugacity Model: | | | |
|---|---|---|---|
| | Mass Amount (%) | Half-Life (hr) | Emissions (kg/hr) |
| | | | |
| Air | 3.16 | 43.4 | 1000 |
| Water | 46.3 | 208 | 1000 |
| Soil | 50.5 | 208 | 1000 |
| Sediment | 0.0691 | 832 | 0 |
| | | | |
| Persistence Time: 222 hr | | | |

- **Biodegradation**

Value 50% degradation after 5 days and 67% after 20 days.
Breakdown Product: It is to be expected that L(+) lactic acid will be taken up into the metabolism of the bacteria in the activated sludge. Degradation products will therefor be $CO_2$ and $H_2O$.
Method: BOD (Biochemical Oxygen Demand) and COD (Chemical Oxygen Demand) determinations were carried out for L(+) lactic acid usig the method described in teh Dutch guidenlines "water determination of biochemical oxgen demand after n days (BODn)" (NEN 6634) and "Water determination of chemical oxygen demand (COD" (NEN 6633) respectively, these methods are similar to those referred in the EC Test Guidelines C.8 and C.9 Two concentrations (2 mg/L and 4 mg/L) were tested.
An oculum was prepared from activated sludge. Its microbial activity appeared to be sufficient although the control substance glucose and glutamic acid had a BOD5 of slightly less than the required value of 4.00 ± 0.75 mg $O_2$/L

## *References Chapter 2: Environmental fate and pathways*


- Lactic Acid: properties and chemistry of lactic acid and derivatives by C.H. Holten, 1971, page 38

- TNO report R 92/018: BOD and COd of L(+) lactic acid according to EC test guidelines C.8 and C.9

- The ecotoxicity and the biodegradability of lactic acid, alkyl esters and lactate salts
  C.T. Bowmer et.al.
  Chemosphere, volume 37, No 7, pp 1317 - 1333, 1998

- Stability of lactic acid and glycolic acid in aqueous systems subjected to acid hydrolysis and thermal decomposition.
  M.M. de Villiers et.al.
  Journal of the society of cosmetic chemists, 48, 165-174 (August 1998)

- Handbook of Chemistry and Physics
  WEAST
  66th Edition

# Chapter 3 Ecotoxicity

- ***Acute/Prolonged Toxicity to Fish***

| | | |
|---|---|---|
| - | Species: | Brachydanio rerio (2.1 ±0.17 cm and 0.07 ±0.018 g) |
| | Report number: | TNO R 91/295 |
| | Type: | Semistatic |
| | Exposure period: | 48 hour(s) |
| | Test substance: | 80% L(+) Lactic Acid |
| | Nominal concentrations: | 0, 100, 180, 320, 560 and 1000 mg/l |
| | Analytical monitoring: | yes |
| | 48h LC50: | = 320 - calculated |
| | Limit Test: | no |
| | Method: | OECD Guideline 203 "Fish, Acute Toxicity Test" |
| | Water temperature: | Between 24.0 and 24.7 °C |
| | Water hardness: | About 220 mg/l expressed as $CaCO_3$. |
| | Alkalinity: | Unknown |
| | Total Organic content: | 2.0 mg/l |
| | Dissolved oxygen: | > 7.3 mg/l |
| | pH levels: | During test between 3.2 and 8.2 |
| | Year: | 1992 |
| | GLP: | yes |
| | Remark: | test solutions are not neutralised. It is more than likely that the low pH value affected the survival of the fishes. |

- Species: Brachydanio rerio (2.1 ±0.17 cm and 0.07 ±0.018 g)
  Report number: TNO R 91/295
  Type: Semistatic
  Exposure period: 96 hour(s)
  Test substance: 80% L(+) Lactic Acid
  Nominal concentrations: 0, 100, 180, 320, 560 and 1000 mg/l
  Analytical monitoring: yes
  96h NOEC: = 320 mg/l - measured/nominal
  96h LC50: = 320 mg/l - calculated
  96h LC100: = 560 mg/l - calculated
  Method: OECD Guideline 203 "Fish, Acute Toxicity Test"
  Water temperature: Between 24.0 and 24.7 °C
  Water hardness: About 220 mg/l expressed as $CaCO_3$.
  Alkalinity: Unknown
  Total Organic content: 2.0 mg/l
  Dissolved oxygen: > 7.3 mg/l
  pH levels: During test between 3.2 and 8.1
  Year: 1992
  GLP: yes
  Remark: test solutions are not neutralised. It is more than likely that the low pH value affected the survival of the fishes.

- Species: Lepomis macrochirus (24± 2.3 mm and 0.37 ± 0.15 g)
  Report number: Analytical Bio Chemistry lab # 32146
  Type: Static
  Exposure period: 96 hour(s)
  Test substance: 80% L(+) Lactic Acid
  Nominal concentrations: 0, 56, 100, 180, 320 and 560 mg/l
  Analytical monitoring: yes
  NOEC: = 56 mg/l - measured/nominal
  24 h LC50: = 140 mg/l - measured/nominal
  48 h LC50: = 130 mg/l - measured/nominal
  96h LC50: = 130 mg/l - measured/nominal
  Limit Test: yes
  Method: :
  Ten fish were exposed to each test concentration and control.
  The procedures for static bioassay described in (1) and (2) below were used in this experiment.
  (1) Committee on methods for Toxicity Tests with Aquatic Organisms (C.E. Stephan chairman). 1975. Methods for acute toxicity tests with fish, macro invertebrates and amphibians.
  Environmental Protection Agency, Ecological Research Series EPA 660/3-75-009, April 1975; 61p
  Water temperature: 22 °C ( ±1,0 )
  Water hardness: 40 - 45 mg/l as $CaCO_3$.
  Alkalinity: 30 - 35 mg/l as $CaCO_3$.
  Dissolved oxygen: 9.0 mg/l (at time = zero)
  pH levels: During test between 7.2 – 7.6

  (2) American Public Health Association. 1980. Standard methods for the examination of water and wastewater. 15th ed. Washington DC 1134p.
  Year: 1984
  GLP: yes

| | |
|---|---|
| Species: | Salmo gairdneri (42 ± 3.4 mm and 1.09 ± 0.28 g) |
| Report number: | Analytical Bio Chemistry lab # 32147 |
| Type: | Static |
| Exposure period: | 96 hour(s) |
| Test substance: | 80% L(+) Lactic Acid |
| Nominal concentratins: | 0, 32, 56, 100, 180 and 320 mg/l |
| Analytical monitoring: | yes |
| NOEC: | = 56 mg/l - measured/nominal |
| 24h LC50: | = 150 mg/l - measured/nominal |
| 48 h LC50: | = 130 mg/l - measured/nominal |
| 96h LC50: | = 130 mg/l - measured/nominal |

Method:

The static fish bioassay was conducted in five gallon glass vessels containing 15 litres reconstituted water. The study was conducted at the nominal concentrations of 32, 56, 100,180 and 320 mg/l. Ten fish were exposed to each test concentration and control.

| | |
|---|---|
| Water temperature: | 12 °C ( ±1.0 ) |
| Water hardness: | 40 - 45 mg/l as $CaCO_3$. |
| Alkalinity: | 30 - 35 mg/l as $CaCO_3$. |
| Dissolved oxygen: | During test between 6.1 – 9.2 mg/l |
| pH levels: | During test between 7.2 – 7.6 |

- ***Acute Toxicity to Aquatic Invertebrates***

| | |
|---|---|
| - Species: | Daphnia magna (less than 24 hours old) |
| Report number: | TNO report R 91/294 |
| Type: | Static |
| Exposure period: | 48 hour(s) |
| Test substance: | 80% L(+) Lactic Acid |
| Nominal concentrations: | 0, 32, 56, 100, 180, 320 and 560 mg/l |
| Analytical monitoring: | yes |
| 48h NOEC: | = 180 mg/l - calculated |
| 48h EC50: | = 240 mg/l - calculated |
| 48h EC100: | = 320 mg/l - calculated |
| 24h EC 50: | = 240mg /l - calculated |
| Limit Test: | no |
| Method**:** | OECD Guide-line 202 |
| Water temperature: | 19.9 °C ( ±1 ) |
| Water hardness: | 220 mg/l as $CaCO_3$. |
| Alkalinity: | Unknown |
| Total organic carbon: | 2.0 mg/l |
| Dissolved oxygen: | > 7.9 mg/l |
| pH levels: | During test between 3.6 – 8.2 |
| Year: | 1992 |
| GLP: | yes |

Remark:
test solutions are not neutralised. It is more than likely that the low pH values affected the mobility of the daphnia's.

- Species: Daphnia magna (less than 24 hours old)
  Report number: Analytical Bio Chemistry lab # 32148
  Type: Static
  Exposure period: 48 hour(s)
  Test substance: 80% L(+) Lactic Acid
  Nominal concentations: 0, 100, 180, 320, 560 and 1000 mg/l
  Analytical monitoring: yes
  NOEC: = 320 mg/l - measured/nominal
  LC50 48h : = 750 mg/l - measured/nominal
  Limit Test: yes

  Method:
  Five concentrations in duplicate of the test compound with ten Daphnia per 250 ml glass beaker were used. The concentrations were a logarithmic series ranging from 100 to 1000 mg/l and included a control. The procedure for static bioassay as described in (1) and (2) below were used.

  (1) Methods of acute toxicity with fish, Macro invertebrates and Amphibians. Stephan, CE, chairman. 1975.
  Committee on Methods for toxicity tests with aquatic organisms. US EPA Ecol. Res. Ser. 660/3-75009.
  (2) American Public Health Association. 1980. Standard methods for the examination of Water and wastewater. 15th ed. Washington DC. 1134p.
  Water temperature: 20 °C ( ±2 )
  Water hardness: 225 - 275 mg/l as $CaCO_3$.
  Alkalinity: 325 - 375 mg/l as $CaCO_3$.
  Dissolved oxygen: 7.1 – 8.5 mg/l (77 – 92 percent saturation at 20°C)
  pH levels: During test between 4.3 – 8.5
  Year 1984
  GLP: yes

- ***Toxicity to Aquatic Plants e.g. Algae***

- Report number: TNO 92/009
  Species: Selenastrum capricornutum (Algae)
  Endpoint: growth rate
  Exposure period: 70 hour(s)
  Test substance**:** 80% L(+) neutralised L(+) lactic acid
  Analytical monitoring: yes
  NOEC: = 1.9 mg/l - calculated
  EC10: = 2.3 mg/l - calculated
  EC50: = 3.5 mg/l - calculated
  EC90 : = 5.4 mg/l - calculated
  Method: OECD Guideline 201 "Algae, Growth Inhibition Test"
  Water temperature: 23 °C ( ±1 )
  Lighting conditions: 120 ± 20% µmol.$S^{-1}$.$m^{-2}$.
  Composition of Growth medium:

| | |
|---|---|
| $NH_4Cl$ | 15 mg/l |
| $MgCL_2.6H_2O$ | 12 mg/l |
| $CaCl_2.2H_2O$ | 18 mg/l |
| $MgSO_4.7H_2O$ | 15 mg/l |
| $KH_2PO_4$ | 1.6 mg/l |
| $FeCl_3.6H_2O$ | 80 µg/l |
| $Na_2EDTA.2H_2O$ | 100 µg/l |
| $H_3BO_3$ | 185 µg/l |
| $MnCl_2.4H_2O$ | 415 µg/l |
| $ZnCl_3$ | 3 µg/l |
| $CoCl_2.6H_2O$ | 1.5 µg/l |
| $CuCl_2.2H_2O$ | 0.01 µg/l |
| $Na_2MoO_4.2H_2O$ | 7 µg/l |
| $NaHCO_3$ | 150 µg/l |

  The pH of this medium after equilibration with air is approximately 8.

  Water hardness: Not applicable
  Dissolved oxygen: flasks were shaken (100 rpm); no data on dissolved oxygen.
  pH levels: During test between 3.6 – 8.2
  Year: 1992
  GLP: yes

- ***Toxicity to Micro-organisms e.g. Bacteria***

- Type: other: laboratory incubations
  Species: Escherichia coli (Bacteria)
  Exposure period: 20 minute(s)
  EC100 : = 15 - measured/nominal
  Test substance`: combinations of 1.0-1.5% lactic acid with 0.1% sodium benzoate, or 0.1% hydrogen peroxide, or 0.005% glycerol monolaurate.
  Result:
  At 22C complete inactivation of E. coli O157:H7 was observed after 20 min. of exposure to 1.5% lactic acid plus 0.1% hydrogen peroxide.
  Conclusion:
  The mentioned treatment could potentially be used to inactivate or reduce E. coli O157:H7 populations on raw products

- Type: other: laboratory incubations on lean beef muscle discs
  Species: other bacteria: Listeria monoccytogenes, Yersinia enterocolitica, Salmonella typhimurium, E.coli, Campylobacter jejuni, Staphylococcus aureus, Pseudomonas fragi, Brochotrix thermosphacta.
  Exposure period: 0 minute(s)
  Remark: Acid temperature (20 & 50 C) and concentration (1%, 3%) and initial numbers of contaminating bacteria (log CFU/cm2 of 3-6) were the variables studied.
  Result: The bactericidal efficacy of lactic acid was often distinct for each organism. Bacterial numbers were maximally reduced with 3% acid at 55C. S.aureus: 1.4 log cycle; P. fragi: 2.3 log cycle; B. thermosphacta: 2.8 log cycle reduction.

# *References Chapter 3: Ecotoxicity*

- Acute / Prolonged toxicity to fish


- TNO report R 91/295; The acute toxicity of L(+)-lactic acid to Brachydanio Rerio (OECD 203).

- Acute toxicity of L(+) lactic acid to Rainbow Trout (Salmo Gairdneri)
  Analytical Biochemistry Laboratories Inc.
  Columbia, MO
  1984

- Acute toxicity of L(+) lactic acid to Bluegill Sunfish (Lepomis macrochirus)
  Analytical Biochemistry Laboratories Inc.
  Columbia, MO
  1984


- Acute toxicity to aquatic invertebrates


- TNO-report R 91/ 294; The acute toxicity of L(+)-lactic acid to Daphnia Magna (OECD 202, 48h).

- Acute toxicity of L(+) lactic acid to Daphnia Magna
  Analytical Biochemistry Laboratories Inc.
  Columbia, MO
  1984


- Toxicity to aquatic plants e.g. algae


- TNO report R 92/009 ; Effect of L(+)-lactic acid on the growth of the alga Selenastrum Capricornutum (OECD 201).


- Toxicity to micro-organisms e.g. bacteria


- Food Microbiology 16: 75-82 (1999), Venkitanarayanan K.S.,
  Zhao T., Doyle M.P., "Inactivation of E.coli 0157:H7 by combinations of GRAS chemicals and temperature".

- Greer G.G. and Dilts B.D., Factors affecting the susceptibility of meat borne pathogens and spoilage bacteria to organic acids.
  Food Research International 25: 355-362 (1992).

## 1. Toxicokinetics, Metabolism and Distribution

- (L)-lactic acid is a natural functional metabolite in mammal, as mammalian fuel.
  According to the lactate shuttle concept, L-lactate represents a major means of distributing carbohydrate potential energy for oxidation and gluconeogenesis. The concept of a "lactate shuttle" (Brooks, 1998) is that during hard exercise, as well as other conditions of accelerated glycolysis, glycolic flux in muscle involves L-lactate formation regardless of the state of oxygenation. The production rate of endogenous (L)-lactate in the resting human is about 1.3 mol (70 kg/bw).24 h-1 (= 117 g/day).

## 2. Acute oral toxicity

**LD50**

| | |
|---|---|
| Title | Acute oral LD50 study in rats |
| Species: | Charles River male/female rats |
| Report number | 410-1369 |
| Year | 1984 |
| GLP | Yes |
| Test substance & purity | L(+) lactic acid 80%, diluted with water. |
| Exposure / dosage | Oral dosage; 3,162 / 3,548 / 3,981 / 4,467 / 5,012 / 5,623 / 6,310 mg /kg bw |
| Exposure Time | One dosage |
| No. of Animals | 55 |
| Experimental design | Method: EPA OPP 81-1 |
| Observations | The following mortalities were observed during the main study testing:<br>dosage mg/kg: 3,162 3,548 3,981 4,467 5,012 5,623 6,310<br><br>males -- -- -- 1/5 3/5 4/5 5/5<br>females 1/5 2/5 5/5 5/5 5/5 5/5 5/5<br><br>All mortalities occurred after dosing on day 0 or in morning of day 1, except for one female dosed at 3,162 mg/kg that was found dead on the morning of day 2. The animals were sacraficed after 14-day observation.<br>Consistent body weight gains were observed on days 7 and 14 for all surviving study animals.<br>Lethargy, ataxia, prostration, irregular breathing, piloerection, squinting, lacrimatiobn, salvation, crusty eyes and muzzle, loose stools, damp or yellow/brown stained fur, and moribund were abnormal clinical signs observed as early as 0 – 1 hour after dosing and as late as day 2. No other abnormal clinical signs were observed during the study.<br><br>Abnormal necropsy findings were observed for all found dead animals and for the 4 surviving females dosed at 3,162 mg/kg.<br>Abnormalities observed during necropsy of found dead animals included: discolored lungs; firm texture of lungs; green foci on one lung; erosion of stomachs; dark, black, brown and/or fluid contents of stomachs; black and/or brown discolored stomachs; a distended stomach with white mucosa; mucosal sloughing, ulceration and hemorrhage of the stomachs; discolored livers; white foci on livers; pale capsular areas, superficial erosion, or mottled livers; a discolored diaphraghm; green-black or brown-black discolored kidneys; and red-brown exudate in the nasal and/or oral regions.<br>Mottled lungs were observed during necropsy of 3 surviving animales dosed at 3,162 mg/kg and thickened stomahcs were also observed during necropsy of 2 surviving animals of the same group. No other abnormalities were observed during necropsy of all study animals. |
| Calculation of LD50 | The oral LD50 value, the 95% confidence interval, the slope of the response curve, and correction factors for 0 and 100% observed responses were calculated for each sex using a method adapted from Litchfield and Wilcoxon.[1] Dose-reponse curves were prepared using the calculated LD50 data. |
| Conclusion | $LD_{50}$ between 3543 and 4936 mg/kg bw |
| Remark | |
| | |

[1] Lichtfield, J.T., Jr. and Wilcoxon, F., „A simplified method of evaluating Dose-effect experiments“, Journal of Pharmacology and Experimental Therapeutics, vol 96, 1949, pages 99-113.

**LD100**

| | |
|---|---|
| Title | LD 100 |
| Species: | 10 rats |
| Concentration: | Oral dosage; dose was daily increased: 0.25 ml till 4.5 ml lactic acid 50% |
| Report number | |
| Year | |
| GLP | |
| Test substance & purity | L(+) lactic acid 80% water |
| Exposure | |
| Exposure Time | |
| No. of Animals | |
| Experimental design | |
| Observations | 2 Rats died after dosing with 3 ml = 7500 mg/kg bw. The animals had a 15% reduction in bw in 1 week. A single administration of large doses did not result in changes in carbon dioxide content or pH of the blood, but a considerable decrease in the pH of the urine. |
| Conclusion | $LD_{100}$: = 11250 mg/kg bw |
| Remark | |
| | |

**Acute toxicity**

| | |
|---|---|
| Title | Acute oral toxicity study in rats |
| Species: | Rats, Charles River |
| Report number | 410-1353 |
| Year | 1983 |
| GLP | Yes |
| Test substance & purity | L(+) lactic acid 80%, diluted with water |
| Exposure | 5 mg / kg bodyweight |
| Exposure Time | One dosage |
| No. of Animals | 10, 5 male and 5 female |
| Experimental design | EPA OPP 81-1. The duration of the study was 14 days. The animals were fasted overnight. The following morning, body weights were recorded, doses were calculated and a measured volume of test article was delivered to each animal by gavage in a single dose. Diet was returend to each animal approx 4 hours after test article administration. Observations for mortality and abnormal clinical signs were done twice daily. Body weights were recorded prior to test article administration on day 0, on day 7 and prior to sacrifice on day 14. Also final body weight were recorded prior to necropsy for animals found dead. |
| Observations | Body weight gains were observed for 3 surviving males and a small weight loss was observed for the fourth surviving animal. Body weight loss was observed for all the animals that were found dead. Lethargy and salivation were observed for all animals and crusty muzzle was observed for 9 animals on the day of dosing and as late as day 9 for one female. Other abnormal clinical signs observed for some animals on the day of dosing and as late as day 2 included ataxia, prostration, irregular breathing, noisy breathing, squinting, lacrimation, crusty eyes, crusty nose and body cool to touch. Other abnormal clinical signs observed prior to death of one female on day 10 included yellow/brown stained fur in the perianal region, abdomen swollen, no stools, and few stools.<br>No other abnormal clinical signs were seen during the study. |
| Conclusion | Four males survived the 14-day duration of the study. One male and all females were found dead on the day of dosing (day 0), on day 1 or on day 10. |
| Remark | Test was done to establish clinical signs after single dose treatment |

## 3. Acute Inhalation Toxicity

**Acute Inhalation toxicity**

| | |
|---|---|
| Title | Acute Inhalation toxicity study |
| Species: | Rat Fischer 344 male/female |
| Concentration: | |
| Report number | I-7083.112 |
| Year | 1987 |
| GLP | Yes |
| Test substance & purity | |
| Exposure | Aerosol containing 7,94 mg/L (nose only) a second group was exposed to air alone. |
| Exposure Time | 4 hour(s) |
| No. of Animals | 10 |
| Experimental design | EPA OPP 81-3; The animals were observed for mortality and pharmacotoxic signs after exposure, at 1 and 3 hours following exposure and once daily after that for 14 days. Complete necropsis were performed on all animals on day 15 of the study. Histopathology was not performed |
| Observations | Animals were observed during exposure for signs of toxicity. Rapid breathing and eye tearing was observed in the treatment group. One and three hours after exposure, the treated and control groups had a hunched posture, red stained fur surrounding the eyes(tearing), ruffled fur, and appeared ungroomed with solid fur. Female rats exposed to the test substance appeared lethargic at one (2/5) and three hours (5/5). The two female rats that were lethargic afte 1 hour also had a rapid, shallow breathing and appeared to be gasping afte one and three hours. By 24 hours, most animals appeared normal and no unusual behavious or appearance was observed for the remainder of the test period. However, of the treated female rats 4/5 hd ruffled, ungroomed fur at 24 hours, and 3/5 had ruffled, ungroomed fur 2, 3 and 4 days after treatment. One female from the treatment group had hunched posture, rapid and shallow breathing, and slight tremors, but these signs were observed on day 5 post-treatment. One female from the treament group died on day 8 post-treatment. No gross lesions were observed at necropsy. |
| Conclusion | Based on these results, the LC50 for L(+)Lactic Acid is greater than 7,94 mg/L. |
| Remark | |

## 4. Acute Dermal Toxicity

- Type: Dermal LD50
  Species: rabbit
  Value: > 2000 mg/kg bw
  Method: OECD Guide-line 402 "Acute dermal Toxicity"

**Acute dermal toxicity**

| | |
|---|---|
| Title | Acute dermal toxicity study in rabbits |
| Species: | Rabbits, New Zeeland white, male and female |
| Report number | 410-1354 |
| Year | 1983 |
| GLP | Yes |
| Test substance & purity | L(+) lactic acid 80%, diluted with water |
| Exposure | 2 g/kg for 2 hours of exposure |
| Exposure Time | 2 g/kg for 2 hours of exposure |
| No. of Animals | 10 |
| Experimental design | EPA OPP 81-2; Approx 24 hours after clipping the skin were abraided sufficiently deep to penetrate the stratum corneum but not the dermis. After test substance application, the trunk of each animal was wrapped. After a 24 hour exposure period, each binder was removed and the test site of the animal was wiped to remove the remaing test article.<br>Animals were observed for mortality and abnormal clinical signs hourly after dosing on day 0. Observations for mortality and abnormal clinical signs were done twice daily thereafter during the duration fo the study (14 days). On day 14 all animals were rendered unconscious and were exsanguinated prior to gross necropsy. |
| Observations | All animals survived the 14-days duration of the study and gained body weight. No abnormal clinical signs were observed during the study. Severe erythema and severe oedema were observed at the test sites of all animals after removal on day 1. Erythema decreased in severity for 3 animals on day 12 or 14, and was not observed for one female on day 14.<br>Oedema decreased in severity for 8 animals and was not observed for one female on day 12 and for one male on day 14. Other dermal reactions observed at test sites included: blanching, necrosis, eschar formation, atonia, desquamation and denuded areas.<br>Other dermal reactions observed at test site included:<br>Blanching, necrosis, eschar formation, eschar peeled off, atonia, desquamation, fissures, denuded areas along abraised lines.<br>Brown, crusted and raised discolorations of the treated skin were observed during necropsy of 3 males and 3 females. Multiple depressions in the treated skin were observed during necropsy of one of the same males, of 2 other males , and of one other female. A dark red focus was observed on the lung of one male. No other abnormalities were observed during necropsy of all males and 4 females, and no abnormalities were observed during necropsy of one female. |
| Conclusion | Dermal LD50 >2000 mg/kg in rabbits |
| Remark | |
| | |

## 5. Skin Irritation

| | |
|---|---|
| Title | Acute dermal irritation/corrosion test with lactic acid (88%) in albino rabbits |
| Species: | Rabbit |
| Concentration: | 88 % |
| Report number | V 86.016 |
| Year | 1986 |
| GLP | Yes |
| Test substance & purity | L(+) lactic acid 88%, diluted with water |
| Exposure | Occlusive |
| Exposure Time | 4 hour(s) |
| No. of Animals | 12 |
| Experimental design | OECD Guide-line 404 "Acute Dermal Irritation/Corrosion"; One day prior to the experiment the hair was removed form the back and flanks of the animals. Six rabbits were treated on the intact skin and on the abraided skin. The abraisions were minor incisions of the stratum corneum, but not sufficient to disturb the underlying derma or to produce bleeding. An amount of 0,5 ml of the test material was brought onto the intact and the abraided skin under a surgical patch measuring 1 inch x 1 inch.<br>After an exposure period of 4 hours the patches and the material applied were removed and the resulting skin reactions were evaluated by the method of Draize et.al. (J. Pharmacol. Exptl. Therap. 82 (1944) 377-390). |
| Observations | After 4 hours the dermal effects generally oserved in all rabbits concisted of very slight to slight ischemic necrosis, moderate to severe haemorrhages and slight or moderate oedema. After 28 hours the dermal effects observed generally consisted of very slight to slight ischemic necrosis, moderate haemorrhages, slight or moderate incrustation and slight oedema.<br>During the course of the following two days ischemci necrosis, haemorrhages and oedema were no longer observed. The application sites generally became crater-shaped with a central sunken area which was moderately or severely encrusted, and a surrounding, raised border of non-necrotic skin showing well-defined erythema.<br>After 7 days this picture had hardly changed, apart from clearance of erythema. The central sunken areas of the application sites generally showed moderate to severe incrustation.<br>At the end of the observation period, after 3 weeks, soms signs of healing were observed at the edges of the encrusted skin areas which had been in contact with the tst material. In the new skin visible under the crust edges coming off from the treated skin, formation of scar tissue could be observed whereas hairgrowth was absent. There were no differences between reactions of the intact skin and those of abraided skin. This scar tissue formed already or to be formed is not considered a reversible skin alteration. |
| Conclusion | On the basis of the results obtained, it can be concluded that lactic acid (88%) is severely irritating to the skin of albino rabbits and that tlactic acid (88%) is corrosive to the skin of albino rabbits. |
| Remark | Other studies have shown that the skin of albino rabbit is not the appropriate animal model when addressing the effects of lactic acid on human skin. This result is therefore not used for the classification. |
| | |

| | |
|---|---|
| Title | Acute dermal irritation / corrosion study with PURAC BF S36 and PURAC BF S/30 in albino rabbits |
| Species: | New Zealand White albino rabbits |
| Concentration: | buffered lactic acid: BF S36 (38% l.a. + 38% sodium lactate, total 76% |

| | |
|---|---|
| | d.s.). BF S30 (60% l.a. + 20% sodium lactate, total 80% d.s.) |
| Report number | V 96.677 |
| Year | 1996 |
| GLP | Yes |
| Test substance & purity | buffered lactic acid: BF S36 (38% l.a. + 38% sodium lactate, total 76% d.s.). BF S30 (60% l.a. + 20% sodium lactate, total 80% d.s.) |
| Exposure | Occlusive |
| Exposure Time | 4 hours. |
| No. of Animals | 3 |
| Experimental design | Four days rpior to the start of the study, the hair was removed from the back and the flanks of the animals in a way to avoid abrasions. Each rabbit was treated simutanneously with the same compound.<br>An amount of 0,5 ml of each test substance was distributed over a pathc measuring 2.5 x 2.5 cm. The two patches were fixed to the application sites. Subsequently, the entire trunk fo the rabbit was wrapped with self-adhesive tape.<br>After a 4 hour exposure period, the test substance and patches were removed and the test sites were cleanded with moistened tissues.<br>After a hour the resulting skin reactions were evaluated by the method of Draize et al (J. Pharmacol. Exp. Ther. 82 (1944) 377-390). Further skin readings were made at approximately 24, 48 and 72 hours after treatment. |
| Observations | After 1-72 hours after treatment, no signs of skin irritation were observed in any of the three rabbits. |
| Conclusion | On the basis of the results obtained, it was concluded that, udner the conditions fo this study, PURAC BF36 and PURAC BF S30 are not irritating for the skin of rabbits after a 4 hour dermal contact period. |
| Remark | |

| | |
|---|---|
| Title | Primary dermal irritation study in rabbits |
| Species: | New Zealand White albino rabbits |
| Concentration: | 80% |
| Report number | 410-1355 |
| Year | 1983 |
| GLP | Yes |
| Test substance & purity | L(+) lactic acid 80%, diluted with water |
| Exposure | Occlusive |
| Exposure Time | 24 hours |
| No. of Animals | 6, 3 male and 3 female |
| Experimental design | Two test sites were prepared on each side of the spinal column in the thoracic region of each animal by closely clipping the hair.<br>Approximately 24 hours after clipping, 0.5 ml of the test article was applied to each 2.5 cm square surgical gauze patch. 2 Application sites on each animal were abraded with a needle to penetrate the stratum corneum but not the dermis. Four patches containg the test article were then applied to each animal and held in place with gauze wrapping.<br>After 24 hours exposure, the patches were removed. Each test site was gently wiped with gauze sponges moistened with water to remove the remainign test article.<br>The skin conditions of each test site was evaluated for erythema, edema and other lesions at 30 and 60 minutes after test article removal. After 30-60 minutes evaluations all animals were euthanized due to severity fo the dermal reactions observed.<br>EPA OPP 81-5 |
| Observations | - Severe erythema at 10 of the 12 abraded test sites (on 5 animals) and at 7 of the 12 intact test sites on 4 of the same animals. Moderate to severe erythema was seen at 2 remaining abraded and 5 remaining intact test sites.<br>- Severe edema at 11 of 12 intact test sites and at 11 of 12 abraded test |

| | |
|---|---|
| | sites. Slight edema at one abraded test site and one intact test site on one animal.<br>- Blanching at both abraded sites on each animal and at both intact sites at 5 animals.<br>- Yellow-brown color of skin at all sites on 3 animals, at both abraded sites on one animal and at one intact site and both abraded sites on a fifth animal.<br>- Red exudate at one intact site on one animal.<br>- Skin missing at all sites of one animal, at one intact site and both abraded sites on one animal, and at one intact site or one abraded site on 2 other animals.<br>No other dermal reactions were observed at the evaluations done at 30 to 60 minutes after test article removal. No abormal clinical sign were observed and no mortalities occurred prior to sacrifice after 30 – 60 minute evaluations. |
| Remark | Under the definition of CFR 49, 173.136, the product does not need to be classified |
| | |

| | |
|---|---|
| Title | Lactic acid Q88: A skin corrosivity test in guinea pigs |
| Species: | Guinea pigs, Dunkin Hartly strain |
| Concentration: | 88% |
| Report number | 235943 |
| Year | 1986 |
| GLP | Yes |
| Test substance & purity | L(+) lactic acid 88%, diluted with water |
| Exposure | Occlusive |
| Exposure Time | Up to 4 hours |
| No. of Animals | 6 |
| Experimental design | The animals were divided inot 2 groups of 3. The hair was clipped from both flanks 24 hours before patch application. Care was taken to avoid abrading of the skin.<br>In group 1, 0.5 ml of the test material was applied (at two sites) under 2 gauze patches each measuring 2.5 x 2.5 cm. The patches were applied to the intact skin and covered with Micropore tape. The whole trunk was then bound with Elastoplast elactic bandage to give a semi-occlusive covering.<br>Group 2 animals were treated in the same way except that the test material was applied under one patch.<br>For group 1 animals the first patch was removed 3 minutes after application and the skin was jently washed with water to remove any residual test material. Reactions were scored. One hour after application the second patch was removed, the skin washed and reactions scored.<br>The patch for group 2 animals was left in place for 4 hours before removal and scoring.<br>Skin reactions were scored for erythema and eschar formation and for oedema formation at patch removal, 1, 24, 48 and 72 hours after patch removal. |
| Observations | In group 1 ( 3 minutes and 1 hour exposure) no erythema, no eschar formation and no oedema formation was seen.<br>In group 2 ( 4 hours exposure) very slight erythema (barely perceptible) and very slight oedema formation (barely perceptible) was seen at patch removal and after one hour.<br>In group 2 no erythema, no eschar formation and no oedema formation was seen at 24, 48 and 72 hours after patch removal. |
| Conclusion | It is concluded from the test results that lactic acid 88% is not corrosive to guinea pig skin. It is also noted that irritation after 4 hours exposure was transient and limited to very mild erythema. |
| Remark | |
| | |

| | |
|---|---|
| Title | Acute dermal irritation/corrosion study with lactic acid 88% in pigs |
| Species: | Healthy male, young pigs from Large white (GY) x Dutch Landrace (NL) |
| Concentration: | 88% |
| Report number | V 87.405 |
| Year | 1987 |
| GLP | Yes |
| Test substance & purity | L(+) lactic acid 88%, diluted with water |
| Exposure | Dermal, 0,5 ml test material per application site, occlusive. |
| Exposure Time | Up to 4 hours |
| No. of Animals | 3 |
| Experimental design | The hair was removed from the animals. The test material was brought into contact with three small separate areas of intact skin. Each of the application sites was covered with occlusive patch measuring 1 inch x 1 inch.The first patch was removed after 3 minutes, the second after 1 hour and the third after 4 hours. Immediately after removing the patches the treated skin areas were treated with lukewarm water, and one hour later the resulting skin reactions were evaluated by the method of Draize et.al. (J. Pharmacol. Exp. Ther. 82 (1944) 377-390). Further readings were made after 1 day and after 2, 3, 7, 14 and 21 days. |
| Observations | No dermal irritaton responses related to treatment with lactic acid (88%) were observed at the test sites of any animal during the 21-day observation period, etiher after a 3 minute contact epriod, after 60 minutes contact period or after a 4-hour contact period.<br>After one day, superficial wounds were observed at the test site of pig no. 1 treated for 60 minutes and at the test site of pig no. 3 treated for 4 hours. In the same animals, similar injuries were also seen at the non-treated skin. After two days, the same test sites and non-treated skin areas in pigs 1 and 3 showed some slight small crusts. After three days these affects had cleared. These minor injuries were probably caused by shaving along the walls or the floor of the stable. |
| Conclusion | Under the conditions of this test it is concluded that lactic acid (88%) is not a primairy skin irritant to the pig skin. |
| Remark | TNO and other experts believe the pig to be a more appropriate and representative animal model than the albino rabbit, when addressing the effects of lactic acid on human skin. |
| | |

## 6. Eye Irritation

| | |
|---|---|
| Species | rabbit |
| Concentration: | 20 % L(+) lactic acid |
| Year | 1973 |
| GLP | yes |
| Test substance & purity | 20 % L(+) lactic acid in water |
| Experimental design | Journal Officiel de la Republique Francaise procedure; eyes were examined after 1 and 24 h and after 2, 3, 4, and 7 days with fluorescent staining. |
| Observations | In same study also 50% sodium lactate was tested, which is not irritating. Instilled at 20% and 10% provoked significant ocular irritation :Acute Ocular Irritation Index (AOII) was 39.50 resp. 31.17. Only for the 10% dilution these lesions were reversible, 7 days after instillation. |
| Conclusion | irritating |
| Remark | **From public literature, see references.** |
| | |

| | |
|---|---|
| Title | Chicken Enucleated eye test with three samples of lactic acid; an alternative to the Draize eye test. |
| Species: | Male or female chickens (ROSS, spring chickens) were used as eye donor. |
| Concentration: | |
| Report number | V96.157 |
| Year | 1996 |
| GLP | Yes |
| Test substance & purity | - Powder H60 (60% L(+) lactic acid and 40% calcium S lactate)<br>- HS88, 88% L(+) lactic acid in water.<br>- BF S36 L(+) lactic acid; L(+) lactic acid and sodium S lactae. |
| Exposure | Corneal |
| Exposure Time | 10 seconds |
| No. of Animals | 10 eyes |
| Experimental design | Within 2 hours after the kill the heads were carefully disseceted and placed in a superfusion apparatus. The eyes are checked for damages. Corneal thickness was measured. Per test sample three eyes were selected for testing whereas one eye was rinsed with iostonic saline only and served as a control of the experimental conditions.<br>At time t=0 0.03 ml / 0.03 g of the test sample was applied to the eye in such a way that the entire surface of the cornea was bathe with the test material. After a total exposure period of 10 seconds, the corneal surface was rinsed thoroughly with with 20 ml of isotonic saline of ambient temperature. The control and the test eyes were examined for changes in corneal thickness (swelling), corneal opacity and fluorescein retention at 0, 30, 75, 120, 180 and 240 minutes after treatment. |
| Observations | - <u>powder H60</u><br>After treatment the thickness of the cornea of the eyes gradually increased considerably; a maximum mean corneal swelling of 17% was obtained at 240 minutes after treatment. In addition, moderate corneal opacity and moderate fluorescein retention by damaged epithelial cells were observed in the eyes.<br>The categories defined for corneal swelling, corneal opacity and fluorescin retention were II, III and III<br><br>- <u>PURAC lactic acid HS88</u><br>After treatment severe to complete corneal opacity was observed in the three test eyes, which hampered the measurement of corneal thickness |

| | |
|---|---|
| | at the 30, 75 and 120 minutes after treatment. At 180 and 240 minutes after treatment corneal thickness could be measured again and at 240 minutes a maximum corneal swelling of 28% was determined. All three eyes showed severe fluorescein retention by damaged epithelial cells. The categories defined for corneal swelling, corneal opacity and fluorescin retention were III, IV and IV<br><br>- PURAC lactic acid bufered BF S36<br>After treament only a slight increase in cornela thickness was observed. A maximum corneal swelling of 6% was obtained 75 minutes after treatment. Very slight corneal opacity and slight fluorescein retention by damaged epithelial cells were observed in the test eyes. The categories defined for corneal swelling, corneal opacity and fluorescin retention were II, I and II<br><br>- Control eye<br>The control eye did not show any unusual effects. |
| Conclusion | - powder H60<br><br>Irritating to the eyes<br><br>- lactic acid HS88<br><br>Severely irritating to the eyes<br><br>- lactic acid bufered BF S36<br><br>Not irritating to the eye. |
| Remark | The measurement of the cornela swelling in this test guarantees a highly objective parameter, which enables the investigator to discriminate the damaging effects of the test material very precisely, this in contrast to the conventional rabbit test which uses subjetive gross measurement only. |
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|---|---|
| Species: | other |
| Concentration: | 85 % |
| Experimental design | Test condition: in vitro using the Eytex Assay (Avon Products, Inc, 1995). Most of the formulations were tested undiluted. pH of formulations varies from 7.52 to 2.02. |
| Observations | Only formulation with pH 2.02 (face cream with 11.8% lactic acid 85%) was moderate severe irritant. The formulations with pH ≥ 5.3 were minimal irritant. |
| Conclusion | irritating |
| Remark | **From public literature, see references.** |
| | |

## 7. Sensitisation

| | |
|---|---|
| Title | Dermal sensitization study in guinea pigs (Buehler Test) |
| Species: | Female, Hartley guinea pigs |
| Concentration: | L(+) lactic acid 80%, diluted with water |
| Report number | 480-2750 |
| Year | 1986 |
| GLP | Yes |
| Test substance & purity | L(+) lactic acid 80%, diluted with water |
| Exposure | Dermal |
| Exposure Time | Two days for the range finding and 35 days for the main study. |
| No. of Animals | 22 (2 for range finding, 10 for study test group and 10 for study naïve group) |
| Experimental design | The hair on the back or left flank (induction) and/or flank (range finding and challenge) of each animal was closely clipped. A 0.5 m sample of the test article and 0.5 ml samples of 30, 10 or 5 % suspensions of the test article in dionized water were placed on separate patches and applied to a range finding animal. The 100% test article was selected for induction. |
| Observations | Preliminary range-finding trials revealed very slight erythema at the 100% concentration of the test article. No other dermal reactions were noted for the other concentrations (3, 10 and 30%). Therefor the 100% concentration of the test article was utilized in the main study testing for contact dermal sensitization potential.<br>No mortalities occurred and all animals gained body weight. The test article (100%) produced very slight erytheam at 3 sites and very slight edema at 1 site after the first induction. Erythema grades increased in severity after the second induction application. Due to the increase of severity of the reactions, the concentration of the test article was reduced to 30% and the induction site was changed to the left flank. Very slight erythema was noted after the fifth induction application. Grades ranging from very slight to severe erythema were noted from the seventh to the nineth indction applications.<br>After the challenge application the test article (100%) produced grade 4 erythema in up to six test animals and in up to eight control animals. These reactions were considered irritation reactions, not sensitization reactions. The reactgions seen in the control animals at the challenge were similar to the reactions seen for the test group animals and the test article was not considered to be a cotnact dermal sensitizer. |
| Conclusion | The reactions seen (very slight to moderate erythema, very slight to moderate oedema) were considered to be irritant reactions, not sensitive reactions. The test article was not considered to be a dermal sensitiser. |
| Remark | |
| | |

## 8. Repeated Dose Toxicity

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|---|---|
| Type | Sub-chronic |
| Species: | rat |
| Concentration: | 4 ml lactic acid 10% on 20 g of meal |
| Exposure Time | Exposure period: 90 days |
| Experimental design | Route of administration: gavage<br>Control Group: yes, concurrent no treatment<br>Frequency of treatment: every day |

| | |
|---|---|
| Observations | No differences in appearance, gross observations at necropsy, or organ weights were observed between the test and control animals. Changes in blood carbon dioxide were slight. |
| Conclusion | |
| Remark | **From public literature, see references.** |
| | |

| | |
|---|---|
| Type | Sub-chronic |
| Species: | female Sprague-Dawley rat |
| Test substance & purity | formulation (face cream containing 0.25% of lactic acid 85%) |
| Exposure | dermal |
| Exposure Time | 13 weeks treatment daily, 5 days/week<br>Control Group: yes, concurrent no treatment |
| Experimental design | Doses: 886 mg/kg bw |
| Observations | No significant gross observations, with the exception of minimal skin irritation. Absolute brain weight and kidney-to-body weight ratios were increased for test animals. No lesions were observed at necropsy or at microscopic examination. |
| Conclusion | The formulation is safe in terms of cumulative toxicity. Based upon the exaggerated dose level used in this study for skin care products, dermal application is not likely to produce adverse effects under conditions of consumer use.LOAEL: 886 mg/kg |
| Remark | **From public literature, see references.** |
| | |

| | |
|---|---|
| Type | Sub-chronic |
| Species: | Fischer 344 male/female rat |
| Concentration: | experiment I: calcium lactate dissolved in drinking water (up to 5%).<br>experiment II: up to 30% calcium lactate in diet. |
| Test substance & purity | Calcium lactate as a salt of lactic acid. |
| Exposure | Oral; 5, 2.5, 1.25, 0.6, 0.3 % |
| Exposure Time | Exposure period: 13 weeks |
| Experimental design | Control Group: yes, concurrent no treatment |
| Observations | a <10 % decrease in body weight gain. all animals survived. some haematological and biochemical parameters changed, but no severe lesions were found in microscopic examination in the experiment with ca-lactate mixed in the diet, the amount of calcium in the urine was significantly increased. Nephrocalcinosis and degeneration in kidneys observed. Indications that Nephrocalcinosis was dependent on the low Ca/Phosphorus ratio of the synthetic diet. |
| Remark | Lactic acid tested as its Calcium salt. From this study the lactate part is relevant, should be separated from effects of the soluble Calcium intake. **From public literature, see references.** |
| | |

| | |
|---|---|
| Type: | Sub-chronic |
| Species: | Syrian hamster male/female |
| Exposure | Group 1 (control):Diet 1, contains 20% sucrose as carcinogenic diet; pure water to drink. Group 2: diet 1, mixed with 0.057 ml lactic acid 80%; pure water to drink. Group 3: same diet 1, but water containing 0.050% v/v lactic acid |
| Exposure Time | 14 weeks |
| Experimental design | daily ad libitum; animals of groups 2 & 3 ingest same amount lactic acid.<br>Post exposure period: sacrificed and autopsy; also oral cavity (caries incidence)<br>Control Group: yes, concurrent no treatment |

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|---|---|
| Observations | three groups same growth and health. No significant differences were found in the incidence or extent of carious lesions among the three groups. |
| Conclusion | dietary lactic acid did not play any important role in development or progress of dental caries. |
| Remark | pH of diet 1 is 5.55, of diet 2 is 5.12. pH of pure water is 6.8 and of water + lactic acid is 3.1 .<br>**From public literature, see references.** |
| | |

## 9. Genetic Toxicity 'in Vitro'

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|---|---|
| Type: | Ames test |
| Species: | |
| Concentration: | 0.5, 1.0, and 2.0 microliter lactic acid/plate |
| Report number | |
| Year | |
| GLP | |
| Test substance & purity | |
| Exposure | |
| Exposure Time | |
| No. of Animals | |
| Experimental design | Salmonella/microsome test (Ames test) and chromosomal aberration test in vitro<br>reverse mutation assays, and Chinese hamster fibroblast cell line<br>S. typhimurium strains TA97, TA98, TA100, TA104<br>Metabolic activation: with and without |
| Observations | negative |
| Conclusion | |
| Remark | **From public literature, see references.** |
| | |

| | |
|---|---|
| Type: | Chinese hamster ovary K1 cells, chromosomal aberration tests, and the pH relationship of the medium and clastogenic activity was examined. |
| Concentration: | 8-35 mM |
| Experimental design | Cells were maintained in Ham's F12 medium, supplemented with 10% foetal calf serum.<br>Cytotoxic Concentration: 14-35 mM, when pH was <= 5.8<br>Metabolic activation: with and without |
| Observations | When the culture medium was first acidified by the lactic acid dose and then neutralised to pH 6.4 or when medium is containing 30 mM HEPES as buffer, lactic acid was non-clastogenic.<br>Pseudo-positive reactions are seen as a result of non-physiological low pH. |
| Conclusion | lactic acid was non-clastogenic |
| Remark | **From public literature, see references.** |
| | |

| | |
|---|---|
| Type | review on several mutagenicity studies with lactic acid and some lactates. |
| Concentration: | various |
| Experimental design | various<br>Metabolic activation: with and without |
| Conclusion | negative |
| Remark | the result of 11 studies is reviewed<br>**From public literature, see references.** |

## 10. Genetic Toxicity ' in Vivo'

Due to the natural nature of L(+) lactic acid and the relative low contribution of "outside L(+) lactic acid" to the human metabolism, in vivo genotoxicity studies will not be required.

- ***Carcinogenicity***

| | |
|---|---|
| Title | |
| Species: | Fischer 344 male/female rat |
| Concentration: | 2.5 or 5 % Calcium lactate in the drinking water. Mean total Calcium lactate intake for males was 329.4 g, resp. 625.4 g; for females 237.7 g, resp. 412.1 g. |
| GLP | yes |
| Test substance & purity | The Calcium salt of lactic acid was tested |
| Exposure | drinking water daily, ad lib |
| Exposure Time | 2 years |
| Experimental design | Control Group: yes, concurrent no treatment |
| Observations | Autopsy on rats that died during study and those killed at the end. Examination macro-and microscopically for presence of non-neoplastic and neoplastic lesions |
| Conclusion | Negative |
| Remark | Lactic acid tested as its Calcium salt. From this study the lactate intake is relevant, should be separated from the Calcium effects of a soluble Calcium salt. **From public literature, see references.** |
| | |

| | |
|---|---|
| Species: | female rabbit |
| Concentration: | 0.1-0.2 g/kg bw (5 months), and 0.1-0.7 g/kg bw (13 months) |
| Exposure | drinking water twice daily |
| Exposure Time | 5 or 13 months |
| Observations | No tumors were reported after 5 or 16 months. Further details not provided. |
| Conclusion | negative |
| Remark | **From public literature, see references.** |
| | |

## 11. Toxicity to Fertility

The nature of the compound (part of human metabolism) does make toxicity studies to fertility not necessary

## 12. Developmental Toxicity/Teratogenicity

| | |
|---|---|
| Species: | female CD-1 mouse |
| Exposure Time | gestational days 6-15<br>Duration of test: 10 days |
| Experimental design | Administration: gavage<br>Frequency of treatment: daily<br>Doses: 570 mg/kg bw/day<br>Control Group:yes, concurrent no treatment |
| Observations | Lactic acid was neither maternotoxic nor embryofetotoxic when given orally to mice at 570 mg/kg bw/day on gestation days 6-15. |
| Conclusion | NOAEL Maternal Toxicity: >= 570 mg/kg bw<br>NOAEL Teratogenicity: >= 570 ml/kg bw |
| Remark | **From public literature, see references.** |
| | |

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